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(54) Title: HUMAN MONOCLONAL ANTIBODIES TO CTLA-4

(54) Titre: ANTICORPS MONOCLONAUX HUMAINS DIRIGES CONTRE L'ANTIGENE CTLA-4

## (57) Abstract

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Also provided is a cell culture system for assaying T cell stimulation.

## (57) Abrégé

La présente invention concerne des anticorps monoclonaux entièrement humains dirigés contre l'antigène CTLA-4. L'invention concerne également des séquences nucléotidiques codant des molécules d'immunoglobuline à chaînes lourdes et légères, des séquences d'acides aminés contenant des molécules d'immunoglobuline à chaînes lourdes et légères et notamment des séquences de chaînes lourdes et légères contiguës, couvrant les régions déterminant la complémentarité (CDR), spécifiquement depuis l'intérieur de FR1 et/ou CDR1 jusqu'à CDR3 et/ou l'intérieur de FR4. En outre, l'invention concerne des anticorps présentant des propriétés de liaison similaires et des anticorps (ou autres antagonistes) ayant une fonctionnalité similaire en tant qu'anticorps.

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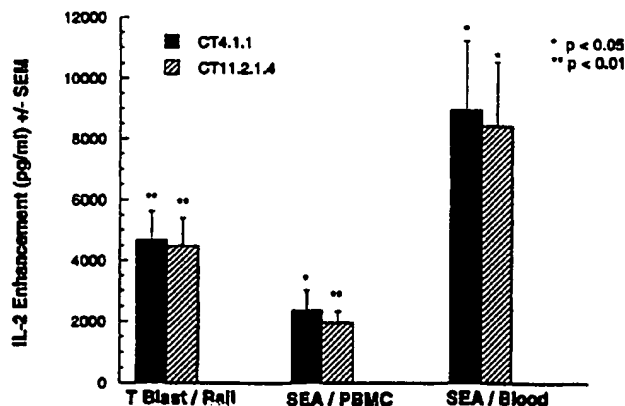


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(21) International Application Number: PCT/US99/30895 (22) International Filing Date: 23 December 1999 (23.12.99) (30) Priority Data: 60/113,647 23 December 1998 (23.12.98) US (71) Applicants (for all designated States except US): PFIZER, INC. [US/US]; Eastpoint Road, Groton, CT 06340 (US). AB- GENIX, INC. [US/US]; 7601 Dumbarton Circle, Fremont, CA 94555 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HANSON, Douglas, Charles [US/US]; 3 Acorn Drive, Niantic, CT 06357 (US). NEVEU, Mark, Joseph [US/US]; 18 Greenbriar Court, Mystic, CT 06355 (US). MUELLER, Eileen, Elliott [US/US]; 4 Butterwick Lane, Old Lyme, CT 06371 (US). HANKE, Jeffrey, Herbert [US/US]; 1 Jefferson Circle, Reading, MA 01867 (US). GILMAN, Steven, Christopher [US/US]; 118 Sill Lane, Old Lyme, CT 06371 (US). DAVIS, C., Geoffrey [US/US]; 1132 Vancouver Avenue, Burlingame, CA 94010 (US). CORVALAN, Jose, Ramon [CL/US]; 125 Williams Lane, Foster City, CA 94010 (US).		(74) Agent: HARE, Christopher, A.; Abgenix, Inc., 7601 Dumbar- ton Circle, Fremont, CA 94555 (US).  (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published With international search report. (88) Date of publication of the international search report: 14 September 2000 (14.09.00)	

(54) Title: HUMAN MONOCLONAL ANTIBODIES TO CTLA-4

Enhancement of IL-2 Production Induced by Anti-CTLA4 MAbs  
(30 µg/ml) in the 72 Hour T Blast / Raji and  
Superantigen Assays (6 Donors)



(57) Abstract

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Also provided is a cell culture system for assaying T cell stimulation.

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**Description**

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## HUMAN MONOCLONAL ANTIBODIES TO CTLA-4

### BACKGROUND OF THE INVENTION

#### 1. Cross-Reference to Related Applications

The present application claims priority to U.S. Provisional Patent Application, Serial No. 60/113,647, filed December 23, 1998, the disclosure of which is hereby incorporated in its entirety herein.

#### 2. Summary of the Invention

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein.

#### 3. Background of the Technology

Regulation of immune response in patients would provide a desirable treatment of many human diseases that could lead to a specificity of action that is rarely found through the use of conventional drugs. Both up-regulation and down-regulation of responses of the immune system would be possible. The roles of T cells and B cells have been extensively studied and characterized in connection with the regulation of immune response. From these studies, the role of T cells appear, in many cases, to be particularly important in disease prevention and treatment.

5 T cells possess very complex systems for controlling their interactions. Interactions between T cells utilize numerous receptors and soluble factors for the process. Thus, what effect any particular signal may have on the immune response generally varies and depends on the particular factors, receptors and  
10 counter-receptors that are involved in the pathway. The pathways for down-regulating responses are as important as those required for activation. Thymic education leading to T-cell tolerance is one mechanism for preventing an immune response to a particular antigen. Other mechanisms, such as secretion of suppressive cytokines, are also known.

10 Activation of T cells requires not only stimulation through the antigen receptor (T cell receptor (TCR)), but additional signaling through co-stimulatory surface molecules such as CD28. The ligands for CD28 are the B7-1 (CD80) and B7-2 (CD86) proteins, which are expressed on antigen-presenting cells such as dendritic cells, activated B-cells or monocytes that interact with T-  
25 cell CD28 or CTLA-4 to deliver a costimulatory signal. The role of costimulatory signaling was studied in experimental allergic encephalomyelitis (EAE) by Perrin et al. *Immunol Res* 14:189-99 (1995). EAE is an autoimmune disorder, induced by Th1 cells directed against myelin antigens that provides an *in vivo* model for studying the role of B7-mediated costimulation in the  
30 induction of a pathological immune response. Using a soluble fusion protein ligand for the B7 receptors, as well as monoclonal antibodies specific for either CD80 or CD86, Perrin et al. demonstrated that B7 costimulation plays a prominent role in determining clinical disease outcome in EAE.

35 The interaction between B7 and CD28 is one of several co-stimulatory signaling pathways that appear to be sufficient to trigger the maturation and proliferation of antigen specific T-cells. Lack of co-stimulation, and the concomitant inadequacy of IL-2 production, prevent subsequent proliferation of the T cell and induce a state of non-reactivity termed "anergy". A variety of  
45 viruses and tumors may block T cell activation and proliferation, leading to insufficient activity or non-reactivity of the host's immune system to the infected or transformed cells. Among a number of possible T-cell disturbances,

anergy may be at least partly responsible for the failure of the host to clear the pathogenic or tumorigenic cells.

The use of the B7 protein to mediate anti-tumor immunity has been described in Chen et al. *Cell* 71:1093-1102 (1992) and Townsend and Allison *Science* 259:368 (1993). Schwartz *Cell* 71:1065 (1992) reviews the role of CD28, CTLA-4, and B7 in IL-2 production and immunotherapy. Harding et al. *Nature* 356:607-609 (1994) demonstrates that CD28 mediated signaling co-stimulates murine T cells and prevents the induction of anergy in T cell clones. See also U.S. Patent Nos. 5,434,131, 5,770,197, and 5,773,253, and International Patent Application Nos. WO 93/00431, WO 95/01994, WO 95/03408, WO 95/24217, and WO 95/33770.

From the foregoing, it was clear that T-cells required two types of signals from the antigen presenting cell (APC) for activation and subsequent differentiation to effector function. First, there is an antigen specific signal generated by interactions between the TCR on the T-cell and MHC molecules presenting peptides on the APC. Second, there is an antigen-independent signal that is mediated by the interaction of CD28 with members of the B7 family (B7-1 (CD80) or B7-2 (CD86)). Exactly where CTLA-4 fit into the milieu of immune responsiveness was initially evasive. Murine CTLA-4 was first identified and cloned by Brunet et al. *Nature* 328:267-270 (1987), as part of a quest for molecules that are preferentially expressed on cytotoxic T lymphocytes. Human CTLA-4 was identified and cloned shortly thereafter by Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988). The murine and human CTLA-4 molecules possess approximately 76% overall sequence homology and approach complete sequence identity in their cytoplasmic domains (Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988)). CTLA-4 is a member of the immunoglobulin (Ig) superfamily of proteins. The Ig superfamily is a group of proteins that share key structural features of either a variable (V) or constant (C) domain of Ig molecules. Members of the Ig superfamily include, but are not limited to, the immunoglobulins themselves, major histocompatibility complex (MHC) class molecules (i.e., MHC class I and II), and TCR molecules.

5 In 1991, Linsley et al. *J. Exp. Med.* 174:561-569 (1991), proposed that  
CTLA-4 was a second receptor for B7. Similarly, Harper et al. *J Immunol*  
10 147:1037-44 (1991) demonstrated that the CTLA-4 and CD28 molecules are  
closely related in both mouse and human as to sequence, message expression,  
5 gene structure, and chromosomal location. See also Balzano et al. *Int J Cancer*  
*Suppl* 7:28-32 (1992). Further evidence of this role arose through functional  
studies. For example, Lenschow et al. *Science* 257:789-792 (1992)  
15 demonstrated that CTLA-4-Ig induced long term survival of pancreatic islet  
grafts. Freeman et al. *Science* 262:907-909 (1993) examined the role of CTLA-  
10 4 in B7 deficient mice. Examination of the ligands for CTLA-4 are described  
in Lenschow et al. *P.N.A.S.* 90:11054-11058 (1993). Linsley et al. *Science*  
257:792-795 (1992) describes immunosuppression *in vivo* by a soluble form of  
CTLA-4. Linsley et al. *J Exp Med* 176:1595-604 (1992) prepared antibodies  
25 that bound CTLA-4 and that were not cross-reactive with CD28 and concluded  
that CTLA-4 is coexpressed with CD28 on activated T lymphocytes and  
cooperatively regulates T cell adhesion and activation by B7. Kuchroo et al.  
*Cell* 80:707-18 (1995) demonstrated that the B7-1 and B7-2 costimulatory  
30 molecules differentially activated the Th1/Th2 developmental pathways. Yi-  
qun et al. *Int Immunol* 8:37-44 (1996) demonstrated that there are differential  
20 requirements for co-stimulatory signals from B7 family members by resting  
versus recently activated memory T cells towards soluble recall antigens. See  
35 also de Boer et al. *Eur J Immunol* 23:3120-5 (1993).

Several groups proposed alternative or distinct receptor/ligand  
interactions for CTLA-4 as compared to CD28 and even proposed a third B-7  
40 25 complex that was recognized by a BB1 antibody. See, for example, Hathcock et  
al. *Science* 262:905-7 (1993), Freeman et al. *Science* 262:907-9 (1993),  
Freeman et al. *J Exp Med* 178:2185-92 (1993), Lenschow et al. *Proc Natl Acad*  
*Sci U S A* 90:11054-8 (1993), Razi-Wolf et al. *Proc Natl Acad Sci U S A*  
45 90:11182-6 (1993), and Boussiotis et al. *Proc Natl Acad Sci U S A* 90:11059-63  
30 (1993). But, see, Freeman et al. *J Immunol* 161:2708-15 (1998) who discuss  
finding that BB1 antibody binds a molecule that is identical to the cell surface  
form of CD74 and, therefore, the BB1 mAb binds to a protein distinct from B7-  
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1, and this epitope is also present on the B7-1 protein. Thus, this observation required the field to reconsider studies using BB1 mAb in the analysis of CD80 expression and function.

Beginning in 1993 and culminating in 1995, investigators began to further delineate the role of CTLA-4 in T-cell stimulation. First, through the use of monoclonal antibodies against CTLA-4, Walunas et al. *Immunity* 1:405-13 (1994) provided evidence that CTLA-4 can function as a negative regulator of T cell activation. Thereafter, Waterhouse et al. *Science* 270:985-988 (1995) demonstrated that mice deficient for CTLA-4 accumulated T cell blasts with up-regulated activation markers in their lymph nodes and spleens. The blast cells also infiltrated liver, heart, lung, and pancreas tissue, and amounts of serum immunoglobulin were elevated and their T cells proliferated spontaneously and strongly when stimulated through the T cell receptor, however, they were sensitive to cell death induced by cross-linking of the Fas receptor and by gamma irradiation. Waterhouse et al. concluded that CTLA-4 acts as a negative regulator of T cell activation and is vital for the control of lymphocyte homeostasis. In a comment in the same issue, Allison and Krummel *Science* 270:932-933 (1995), discussed the work of Waterhouse et al. as demonstrative that CTLA-4 acts to down regulate T-cell responsiveness or has an inhibitory signaling role in T-cell activation and development. Tivol et al. *Immunity* 3:541-7 (1995) also generated CTLA-4-deficient mice and demonstrated that such mice rapidly develop lymphoproliferative disease with multiorgan lymphocytic infiltration and tissue destruction, with particularly severe myocarditis and pancreatitis. They concluded that CTLA-4 plays a key role in down-regulating T cell activation and maintaining immunologic homeostasis. Also, Krummel and Allison *J Exp Med* 182:459-65 (1995) further clarified that CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. They generated an antibody to CTLA-4 and investigated the effects of its binding to CTLA-4 in a system using highly purified T cells. In their report, they showed that the presence of low levels of B7-2 on freshly explanted T cells can partially inhibit T cell proliferation, and this inhibition was mediated by interactions with CTLA-4. Cross-linking of CTLA-4 together with the TCR and CD28 strongly

5 inhibits proliferation and IL-2 secretion by T cells. Finally, the results showed  
that CD28 and CTLA-4 deliver opposing signals that appear to be integrated by  
the T cell in determining the response to antigen. Thus, they concluded that the  
10 outcome of T cell antigen receptor stimulation is regulated by CD28  
costimulatory signals, as well as inhibitory signals derived from CTLA-4. *See*  
also Krummel et al. *Int Immunol* 8:519-23 (1996) and U.S. Patent No.  
5,811,097 and International Patent Application No. WO 97/20574.

15 A variety of additional experiments have been conducted further  
elucidating the above function of CTLA-4. For example, Walunas et al. *J Exp*  
10 *Med* 183:2541-50 (1996), through the use of anti-CTLA-4 antibodies, suggested  
that CTLA-4 signaling does not regulate cell survival or responsiveness to IL-2,  
20 but does inhibit CD28-dependent IL-2 production. Also, Perrin et al. *J Immunol*  
157:1333-6 (1996), demonstrated that anti-CTLA-4 antibodies in experimental  
allergic encephalomyelitis (EAE), exacerbated the disease and enhanced  
25 mortality. Disease exacerbation was associated with enhanced production of the  
encephalitogenic cytokines TNF-alpha, IFN-gamma and IL-2. Thus, they  
concluded that CTLA-4 regulates the intensity of the autoimmune response in  
EAE, attenuating inflammatory cytokine production and clinical disease  
30 manifestations. *See also* Hurwitz et al. *J Neuroimmunol* 73:57-62 (1997) and  
Cepero et al. *J Exp Med* 188:199-204 (1998) (an anti-CTLA-4 hairpin ribozyme  
that specifically abrogates CTLA-4 expression after gene transfer into a murine  
35 T-cell model).

In addition, Blair et al. *J Immunol* 160:12-5 (1998) assessed the  
functional effects of a panel of CTLA-4 monoclonal antibodies (mAbs) on  
40 25 resting human CD4+ T cells. Their results demonstrated that some CTLA-4  
mAbs could inhibit proliferative responses of resting CD4+ cells and cell cycle  
transition from G0 to G1. The inhibitory effects of CTLA-4 were evident within  
4 h, at a time when cell surface CTLA-4 expression remained undetectable.  
45 Other CTLA-4 mAbs, however, had no detectable inhibitory effects, indicating  
30 that binding of mAbs to CTLA-4 alone was not sufficient to mediate down-  
regulation of T cell responses. Interestingly, while IL-2 production was shut off,  
inhibitory anti-CTLA-4 mAbs permitted induction and expression of the cell  
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5 survival gene bcl-X(L). Consistent with this observation, cells remained viable and apoptosis was not detected after CTLA-4 ligation.

10 In connection with anergy, Perez et al. *Immunity* 6:411-7 (1997) demonstrated that the induction of T cell anergy was prevented by blocking  
5 CTLA-4 and concluded that the outcome of antigen recognition by T cells is determined by the interaction of CD28 or CTLA-4 on the T cells with B7 molecules. Also, Van Parijs et al. *J Exp Med* 186:1119-28 (1997) examined the  
15 role of interleukin 12 and costimulators in T cell anergy *in vivo* and found that through inhibiting CTLA-4 engagement during anergy induction, T cell  
10 proliferation was blocked, and full Th1 differentiation was not promoted. However, T cells exposed to tolerogenic antigen in the presence of both IL-12  
20 and anti-CTLA-4 antibody were not energized, and behaved identically to T cells which have encountered immunogenic antigen. These results suggested  
25 that two processes contribute to the induction of anergy *in vivo*: CTLA-4 engagement, which leads to a block in the ability of T cells to proliferate, and  
the absence of a prototypic inflammatory cytokine, IL-12, which prevents the  
differentiation of T cells into Th1 effector cells. The combination of IL-12 and  
30 anti-CTLA-4 antibody was sufficient to convert a normally tolerogenic stimulus to an immunogenic one.

20 In connection with infections, McCoy et al. *J Exp Med* 186:183-7 (1997) demonstrated that anti-CTLA-4 antibodies greatly enhanced and accelerated the  
35 T cell immune response to *Nippostrongylus brasiliensis*, resulting in a profound reduction in adult worm numbers and early termination of parasite egg  
production. See also Murphy et al. *J. Immunol.* 161:4153-4160 (1998)  
40 (*Leishmania donovani*).

45 In connection with cancer, Kwon et al. *PNAS USA* 94:8099-103 (1997) established a syngeneic murine prostate cancer model and examined two  
distinct manipulations intended to elicit an antiprostata cancer response through  
enhanced T cell costimulation: (i) provision of direct costimulation by prostate  
30 cancer cells transduced to express the B7.1 ligand and (ii) *in vivo* antibody-mediated blockade of T cell CTLA-4, which prevents T cell down-regulation. It  
was demonstrated that *in vivo* antibody-mediated blockade of CTLA-4  
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5 enhanced antiprostata cancer immune responses. Also, Yang et al. *Cancer Res*  
57:4036-41 (1997) investigated whether the blockade of the CTLA-4 function  
10 leads to enhancement of antitumor T cell responses at various stages of tumor  
growth. Based on *in vitro* and *in vivo* results they found that CTLA-4 blockade  
5 in tumor-bearing individuals enhanced the capacity to generate antitumor T-cell  
responses, but the expression of such an enhancing effect was restricted to early  
stages of tumor growth in their model. Further, Hurwitz et al. *Proc Natl Acad*  
15 *Sci U S A* 95:10067-71 (1998) investigated the generation of a T cell-mediated  
antitumor response depends on T cell receptor engagement by major  
10 histocompatibility complex/antigen as well as CD28 ligation by B7. Certain  
tumors, such as the SM1 mammary carcinoma, were refractory to anti-CTLA-4  
immunotherapy. Thus, through use of a combination of CTLA-4 blockade and  
20 a vaccine consisting of granulocyte-macrophage colony-stimulating factor-  
expressing SM1 cells, regression of parental SM1 tumors was observed, despite  
25 the ineffectiveness of either treatment alone. This combination therapy resulted  
in long-lasting immunity to SM1 and depended on both CD4(+) and CD8(+) T  
cells. The findings suggested that CTLA-4 blockade acts at the level of a host-  
30 derived antigen-presenting cell.

In connection with diabetes, Luhder et al. *J Exp Med* 187:427-32 (1998)  
20 injected an anti-CTLA-4 mAb into a TCR transgenic mouse model of diabetes  
at different stages of disease. They found that engagement of CTLA-4 at the  
35 time when potentially diabetogenic T cells are first activated is a pivotal event;  
if engagement is permitted, invasion of the islets occurs, but remains quite  
innocuous for months. If not, insulitis is much more aggressive, and diabetes  
25 quickly ensues.

In connection with vaccine immunization, Horspool et al. *J Immunol*  
160:2706-14 (1998) found that intact anti-CTLA-4 mAb but not Fab fragments  
45 suppressed the primary humoral response to pCIA/beta gal without affecting  
recall responses, indicating CTLA-4 activation inhibited Ab production but not  
30 T cell priming. Blockade of the ligands for CD28 and CTLA-4, CD80 (B7-1)  
and CD86 (B7-2), revealed distinct and nonoverlapping function. Blockade of  
50 CD80 at initial immunization completely abrogated primary and secondary Ab

5 responses, whereas blockade of CD86 suppressed primary but not secondary responses. Simultaneous blockade of CD80 + CD86 was less effective at suppressing Ab responses than either alone. Enhancement of costimulation via  
10 coinjection of B7-expressing plasmids augmented CTL responses but not Ab responses, and without evidence of Th1 to Th2 skewing. These findings suggest complex and distinct roles for CD28, CTLA-4, CD80, and CD86 in T cell  
15 costimulation following nucleic acid vaccination.

15 In connection with allograft rejection, Markees et al. *J Clin Invest* 101:2446-55 (1998) found in a mouse model of skin allograft rejection that acceptance initially depended on the presence of IFN-gamma, CTLA-4, and  
20 CD4(+) T cells. Addition of anti-CTLA-4 or anti-IFN-gamma mAb to the protocol was associated with prompt graft rejection, whereas anti-IL-4 mAb had no effect.

25 In connection with the role of CTLA-4 in relation to CD28, Fallarino et al. *J Exp Med* 188:205-10 (1998) generated TCR transgenic/recombinase activating gene 2-deficient/CD28-wild-type or CD28-deficient mice which were immunized with an antigen-expressing tumor. Primed T cells from both types  
30 of mice produced cytokines and proliferated in response to stimulator cells lacking B7 expression. However, whereas the response of CD28+/+ T cells was augmented by costimulation with B7-1, the response of the CD28-/- T cells was strongly inhibited. This inhibition was reversed by monoclonal antibody against  
35 B7-1 or CTLA-4. Thus, CTLA-4 can potently inhibit T cell activation in the absence of CD28, indicating that antagonism of a TCR-mediated signal is sufficient to explain the inhibitory effect of CTLA-4. Also, Lin et al. *J Exp Med* 188:199-204 (1998) studied rejection of heart allografts in CD28-deficient mice.  
40 H-2(q) hearts were transplanted into allogeneic wild-type or CD28-deficient mice (H-2(b)). Graft rejection was delayed in CD28-deficient compared with wild-type mice. Treatment of wild-type recipients with CTLA-4-immunoglobulin (Ig), or with anti-B7-1 plus anti-B7-2 mAbs significantly  
45 prolonged allograft survival. In contrast, treatment of CD28-deficient mice with CTLA-4-Ig, anti-B7-1 plus anti-B7-2 mAbs, or a blocking anti-CTLA-4 mAb induced acceleration of allograft rejection. This increased rate of graft rejection  
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5 was associated with more severe mononuclear cell infiltration and enhanced levels of IFN-gamma and IL-6 transcripts in donor hearts of untreated wild-type and CTLA-4-Ig- or anti-CTLA-4 mAb-treated CD28-deficient mice. Thus, the  
10 negative regulatory role of CTLA-4 extends beyond its potential ability to prevent CD28 activation through ligand competition. Even in the absence of CD28, CTLA-4 plays an inhibitory role in the regulation of allograft rejection.

15 Also, further characterization of the expression of CTLA-4 has been investigated. For example, Alegre et al. *J Immunol* 157:4762-70 (1996) proposed that surface CTLA-4 is rapidly internalized, which may explain the  
20 low levels of expression generally detected on the cell surface. They concluded that both CD28 and IL-2 play important roles in the up-regulation of CTLA-4 expression. In addition, the cell surface accumulation of CTLA-4 appeared to be primarily regulated by its rapid endocytosis. Also, Castan et al. *Immunology* 90:265-71 (1997) based on *in situ* immunohistological analyses of the  
25 expression of CTLA-4, suggested that germinal center T cells, which were CTLA-4 positive, could be important to immune regulation.

30 Accordingly, in view of the broad and pivotal role that CTLA-4 appears to possess in immune responsiveness, it would be desirable to generate antibodies to CTLA-4 that can be utilized effectively in immunotherapy. Moreover, it would be desirable to generate antibodies against CTLA-4 that can  
35 be utilized in chronic diseases in which repeat administrations of the antibodies are required.

#### **BRIEF DESCRIPTION OF THE DRAWING FIGURES**

40 25 Figure 1 provides a series of nucleotide and an amino acid sequences of heavy chain and kappa light chain immunoglobulin molecules in accordance with the invention: 4.1.1 (Figure 1A), 4.8.1 (Figure 1B), 4.14.3 (Figure 1C), 6.1.1 (Figure 1D), 3.1.1 (Figure 1E), 4.10.2 (Figure 1F), 2.1.3 (Figure 1G),  
45 4.13.1 (Figure 1H), 11.2.1 (Figure 1I), 11.6.1 (Figure 1J), 11.7.1 (Figure 1K), 12.3.1.1 (Figure 1L), and 12.9.1.1 (Figure 1M).

50 Figure 2 provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1,

5 4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline  
DP-50 (3-33) amino acid sequence. Differences between the DP-50 germline  
sequence and that of the sequence in the clones are indicated in bold. The  
10 Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of  
5 the antibodies as shaded.

Figure 3 provides a sequence alignment between the predicted heavy  
chain amino acid sequence of the clone 2.1.3 and the germline DP-65 (4-31)  
15 amino acid sequence. Differences between the DP-65 germline sequence and  
that of the sequence in the clone are indicated in bold. The Figure also shows  
10 the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as  
underlined.

Figure 4 provides a sequence alignment between the predicted kappa  
light chain amino acid sequence of the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 4.10.2,  
25 and 4.13.1 and the germline A27 amino acid sequence. Differences between the  
A27 germline sequence and that of the sequence in the clone are indicated in  
bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3  
sequences of the antibody as underlined. Apparent deletions in the CDR1s of  
30 clones 4.8.1, 4.14.3, and 6.1.1 are indicated with "0s".

Figure 5 provides a sequence alignment between the predicted kappa  
20 light chain amino acid sequence of the clones 3.1.1, 11.2.1, 11.6.1, and 11.7.1  
and the germline 012 amino acid sequence. Differences between the 012  
germline sequence and that of the sequence in the clone are indicated in bold.  
35 The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences  
of the antibody as underlined.

Figure 6 provides a sequence alignment between the predicted kappa  
40 light chain amino acid sequence of the clone 2.1.3 and the germline A10/A26  
amino acid sequence. Differences between the A10/A26 germline sequence and  
that of the sequence in the clone are indicated in bold. The Figure also shows  
45 the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as  
underlined.

Figure 7 provides a sequence alignment between the predicted kappa  
50 light chain amino acid sequence of the clone 12.3.1 and the germline A17

5 amino acid sequence. Differences between the A17 germline sequence and that  
of the sequence in the clone are indicated in bold. The Figure also shows the  
positions of the CDR1, CDR2, and CDR3 sequences of the antibody as  
10 underlined.

5 Figure 8 provides a sequence alignment between the predicted kappa  
light chain amino acid sequence of the clone 12.9.1 and the germline A3/A19  
amino acid sequence. Differences between the A3/A19 germline sequence and  
15 that of the sequence in the clone are indicated in bold. The Figure also shows  
the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as  
10 underlined.

20 Figure 9 provides a summary of N-terminal amino acid sequences  
generated through direct protein sequencing of the heavy and light chains of the  
antibodies.

25 Figure 10 provides certain additional characterizing information about  
certain of the antibodies in accordance with the invention. In Figure 10A, data  
related to clones 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.14.3, and 6.1.1 is summarized.  
Data related to concentration, isoelectric focusing (IEF), SDS-PAGE, size  
30 exclusion chromatography, liquid chromatography/mass spectroscopy (LCMS),  
mass spectroscopy (MALDI), light chain N-terminal sequences is provided.  
20 Additional detailed information related to IEF is provided in Figure 10B; related  
to SDS-PAGE is provided in 10C; and SEC of the 4.1.1 antibody in 10D.

35 Figure 11 shows the expression of B7-1 and B7-2 on Raji cells using  
anti-CD80-PE and anti-CD86-PE mAbs.

40 Figure 12 shows the concentration dependent enhancement of IL-2  
25 production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking  
antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1).

45 Figure 13 shows the concentration dependent enhancement of IFN- $\gamma$   
production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking  
antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1)(same donor T cells).

50 Figure 14 shows the mean enhancement of IL-2 production in T cells  
30 from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell  
blast/Raji assay.



Figure 15 shows the mean enhancement of IFN- $\gamma$  production in T cells from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell blast/Raji assay.

Figure 16 shows the enhancement of IL-2 production in hPBMC from 5 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 hours after stimulation with SEA.

Figure 17 shows the enhancement of IL-2 production in whole blood from 3 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 and 96 hours after stimulation with SEA.

Figure 18 shows the inhibition of tumor growth with an anti-murine CTLA-4 antibody in a murine fibrosarcoma tumor model.

Figure 19 shows enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji and Superantigen (whole blood and peripheral blood mononuclear cells from 6 donors) assays.

Figure 20 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji assay.

Figure 21 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour Superantigen whole blood assay stimulated with 100 ng/ml superantigen.

Figure 22 provides a series of additional nucleotide and amino acid sequences of the following anti-CTLA-4 antibody chains: full length 4.1.1 heavy chain (cDNA 22(a), genomic 22(b), and amino acid 22(c)), full length aglycosylated 4.1.1 heavy chain (cDNA 22(d) and amino acid 22(e)), 4.1.1 light chain (cDNA 22(f) and amino acid 22(g)), full length 4.8.1 heavy chain (cDNA 22(h) and amino acid 22(i)), 4.8.1 light chain (cDNA 22(j) and amino acid 22(k)), full length 6.1.1 heavy chain (cDNA 22(l) and amino acid 22(m)), 6.1.1 light chain (cDNA 22(n) and amino acid 22(o)), full length 11.2.1 heavy chain (cDNA 22(p) and amino acid 22(q)), and 11.2.1 light chain (cDNA 22 (r) and amino acid 22(s)).

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided an antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V<sub>H</sub>3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or framework sequences shown in Figure 2. In a preferred embodiment, the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70. In another preferred embodiment, the antibody further comprises a light chain variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

In accordance with a second aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:1 and a light chain variable amino acid sequence comprising SEQ ID NO:14.

In accordance with a third aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:2 and a light chain variable amino acid sequence comprising SEQ ID NO:15.

In accordance with a fourth aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:4 and a light chain variable amino acid sequence comprising SEQ ID NO:17.

5 In accordance with a fifth aspect of the present invention, there is provided an isolated human monoclonal antibody that is capable of binding to CTLA-4. In a preferred embodiment, antibody is capable of competing for  
10 binding with CTLA-4 with an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from  
15 the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian species comprises mouse, rat, and rabbit and more preferably mouse and rat. In another preferred  
20 embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys. In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the  
25 antibody is about  $10^{-9}$  M or greater and preferably about  $10^{-10}$  M or greater. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 100 nM or greater  
30 and preferably lower than about 0.50 nM. In another preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846 pg/ml or greater. In another preferred embodiment, the antibody enhances IFN- $\gamma$  production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233  
35 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2  
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5 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

10 In accordance with a sixth aspect of the present invention, there is  
5 provided a humanized antibody that possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1.1, 4.1.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1,  
15 and 12.9.1.1. In a preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian  
10 species comprises mouse, rat, and rabbit and preferably mouse and rat. In another preferred embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys.  
20 In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and  
25 preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the antibody is about  $10^{-9}$  M or greater and preferably about  $10^{-10}$  M or greater. In another preferred embodiment, the antibody inhibits  
30 binding between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the  
20 antibody inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 100 nM or greater and preferably lower than about 0.50 nM. In another  
35 preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846  
pg/ml or greater. In another preferred embodiment, the antibody enhances IFN-  
40  $\gamma$  production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233 pg/ml or greater. In another preferred embodiment,  
the antibody induces IL-2 production in a hPBMC or whole blood superantigen  
45 assay by about 500 pg/ml or greater. In another preferred embodiment, the  
antibody enhances IL-2 production in a hPBMC or whole blood superantigen  
30 assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

5 In accordance with a seventh aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V<sub>H</sub> 3-33 gene family operably linked in frame with a CDR1, a CDR2, 10 and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figure 2. In a preferred embodiment, the antibody of Claim 32, further comprising any of the somatic mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2. 15

10 In accordance with an eighth aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V<sub>H</sub> 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody has the following properties: a binding 25 affinity for CTLA-4 of about 10<sup>-9</sup> or greater; inhibits binding between CTLA-4 and B7-1 with an IC<sub>50</sub> of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC<sub>50</sub> of about 100 nM or lower; and enhances cytokine production in an assay of human T cells by 500 pg/ml or greater. 30

In accordance with a ninth aspect of the present invention, there is 20 provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3, which antibody has the following properties: a binding affinity for CTLA-4 of 35 about 10<sup>-9</sup> or greater; inhibits binding between CTLA-4 and B7-1 with an IC<sub>50</sub> of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC<sub>50</sub> of about 100 nM or lower; and enhances cytokine production in an assay of 40 human T cells by 500 pg/ml or greater.

45 In accordance with a tenth aspect of the present invention, there is provided a cell culture system for assaying T cell stimulation, comprising a 30 culture of human T cell blasts co-cultured with a Raji cell line. In a preferred embodiment, the T cell blasts are washed prior to culture with the Raji cell line. 50

5 In accordance with an eleventh aspect of the present invention, there is  
provided an assay for measuring T cell stimulation, comprising: providing a  
culture of human T cell blasts and a Raji cell line; contacting the culture with an  
10 agent; and measuring cytokine production by the culture.

5 In accordance with an twelfth aspect of the present invention, there is  
provided a functional assay for screening a moiety for T cell stimulatory  
function, comprising: providing a culture of human T cell blasts and a Raji cell  
15 line; contacting the culture with the moiety; and assessing cytokine production  
by the culture.

10 In accordance with a thirteenth aspect of the present invention, there is  
provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising  
contacting a culture comprising human T cell blasts and a Raji cell line with an  
20 agent and assessing cytokine production by the culture.

25 In accordance with a fourteenth aspect of the present invention, there is  
provided a method for screening an agent for T cell stimulatory activity,  
comprising: contacting the agent with a cell culture comprising human T cell  
blasts and a Raji cell line; and assessing cytokine production by the culture.

30 In each of the tenth through the fourteenth aspects of the present  
invention, in a preferred embodiment, the T cell blasts are washed prior to  
20 culture with the Raji cell line. In another preferred embodiment, the cytokine is  
IL-2 or IFN- $\gamma$ . In a preferred embodiment, cytokine production is measured in  
supernatant isolated from the culture. In a preferred embodiment, the agent is  
35 an antibody and preferably binds to CTLA-4.

40 In accordance with a fifteenth aspect of the present invention, there is  
provided an assay for measuring T cell stimulation, comprising: providing a  
population of human peripheral blood mononuclear cells or human whole blood  
stimulated with staphylococcus enterotoxin A; contacting the culture with an  
agent; and measuring cytokine production by the cell population.

45 In accordance with a sixteenth aspect of the present invention, there is  
provided a functional assay for screening a moiety for T cell stimulatory  
30 function, comprising: providing a population of human peripheral blood  
mononuclear cells or human whole blood stimulated with staphylococcus  
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5 enterotoxin A; contacting the culture with the moiety; and assessing cytokine production by the cell population.

10 In accordance with a seventeenth aspect of the present invention, there is provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising  
5 contacting a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A with an agent and assessing cytokine production by the cell population.

15 In accordance with an eighteenth aspect of the present invention, there is provided a method for screening an agent for T cell stimulatory activity,  
10 comprising: contacting the agent with a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; and assessing cytokine production by the cell population.

20 In each of the fifteenth through the eighteenth aspects of the present invention, in a preferred embodiment, the cytokine is IL-2. In another preferred  
25 embodiment, cytokine production is measured in supernatant isolated from the culture. In a preferred embodiment, the agent is an antibody and preferably binds to CTLA-4.

#### 30 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

20 In accordance with the present invention, there are provided fully human monoclonal antibodies against human CTLA-4. Nucleotide sequences encoding  
35 and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly sequences corresponding to a contiguous heavy and light chain sequences from FR1 and CDR1 through CDR3 and FR4, are provided.  
40 Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Hybridomas expressing such immunoglobulin molecules and  
45 monoclonal antibodies are also provided.

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Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:



5 The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not  
10 associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a  
15 larger sequence.

The term "isolated protein" referred to herein means a protein of cDNA,  
10 recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a  
20 different species, or (4) does not occur in nature.

25 The term "polypeptide" as used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain  
30 immunoglobulin molecules and the human kappa light chain immunoglobulin molecules represented in Figure 1, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as the kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs  
35 thereof.

40 The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism  
45 (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.  
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The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

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The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

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The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

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The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant.

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Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche et al. *Nucl. Acids Res.* 14:9081 (1986); Stec et al. *J. Am. Chem. Soc.* 106:6077 (1984); Stein et al. *Nucl. Acids Res.* 16:3209 (1988); Zon et al. *Anti-Cancer Drug Design* 6:539 (1991); Zon et al. *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec et al. U.S. Patent No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for

5 maximum matching. Gaps (in either of the two sequences being matched) are  
allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or  
less being more preferred. Alternatively and preferably, two protein sequences  
10 (or polypeptide sequences derived from them of at least 30 amino acids in  
5 length) are homologous, as this term is used herein, if they have an alignment  
score of at more than 5 (in standard deviation units) using the program ALIGN  
with the mutation data matrix and a gap penalty of 6 or greater. *See* Dayhoff,  
15 M.O., in *Atlas of Protein Sequence and Structure*, pp. 101-110 (Volume 5,  
National Biomedical Research Foundation (1972)) and Supplement 2 to this  
10 volume, pp. 1-10. The two sequences or parts thereof are more preferably  
homologous if their amino acids are greater than or equal to 50% identical when  
20 optimally aligned using the ALIGN program. The term "corresponds to" is  
used herein to mean that a polynucleotide sequence is homologous (i.e., is  
identical, not strictly evolutionarily related) to all or a portion of a reference  
25 polynucleotide sequence, or that a polypeptide sequence is identical to a  
reference polypeptide sequence. In contradistinction, the term "complementary  
to" is used herein to mean that the complementary sequence is homologous to  
30 all or a portion of a reference polynucleotide sequence. For illustration, the  
nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC"  
20 and is complementary to a reference sequence "GTATA".

35 The following terms are used to describe the sequence relationships  
between two or more polynucleotide or amino acid sequences: "reference  
sequence", "comparison window", "sequence identity", "percentage of sequence  
40 25 identity", and "substantial identity". A "reference sequence" is a defined  
sequence used as a basis for a sequence comparison; a reference sequence may  
be a subset of a larger sequence, for example, as a segment of a full-length  
cDNA or gene sequence given in a sequence listing or may comprise a complete  
45 cDNA or gene sequence. Generally, a reference sequence is at least 18  
30 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8  
amino acids in length, and often at least 48 nucleotides or 16 amino acids in  
length. Since two polynucleotides or amino acid sequences may each (1)

5 comprise a sequence (i.e., a portion of the complete polynucleotide or amino  
acid sequence) that is similar between the two molecules, and (2) may further  
10 comprise a sequence that is divergent between the two polynucleotides or amino  
acid sequences, sequence comparisons between two (or more) molecules are  
5 typically performed by comparing sequences of the two molecules over a  
"comparison window" to identify and compare local regions of sequence  
15 similarity. A "comparison window", as used herein, refers to a conceptual  
segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein  
a polynucleotide sequence or amino acid sequence may be compared to a  
10 reference sequence of at least 18 contiguous nucleotides or 6 amino acid  
sequences and wherein the portion of the polynucleotide sequence in the  
20 comparison window may comprise additions, deletions, substitutions, and the  
like (i.e., gaps) of 20 percent or less as compared to the reference sequence  
(which does not comprise additions or deletions) for optimal alignment of the  
25 two sequences. Optimal alignment of sequences for aligning a comparison  
window may be conducted by the local homology algorithm of Smith and  
Waterman *Adv. Appl. Math.* 2:482 (1981), by the homology alignment  
30 algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search  
for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)*  
20 85:2444 (1988), by computerized implementations of these algorithms (GAP,  
BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package  
35 Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.),  
Geneworks, or MacVector software packages), or by inspection, and the best  
alignment (i.e., resulting in the highest percentage of homology over the  
40 comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide or amino  
45 acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-  
residue basis) over the comparison window. The term "percentage of sequence  
30 identity" is calculated by comparing two optimally aligned sequences over the  
window of comparison, determining the number of positions at which the  
50 identical nucleic acid base (e.g., A, T, C, G, U, or I) or residue occurs in both

5 sequences to yield the number of matched positions, dividing the number of  
matched positions by the total number of positions in the comparison window  
(i.e., the window size), and multiplying the result by 100 to yield the percentage  
10 of sequence identity. The terms "substantial identity" as used herein denotes a  
5 characteristic of a polynucleotide or amino acid sequence, wherein the  
polynucleotide or amino acid comprises a sequence that has at least 85 percent  
sequence identity, preferably at least 90 to 95 percent sequence identity, more  
15 usually at least 99 percent sequence identity as compared to a reference  
sequence over a comparison window of at least 18 nucleotide (6 amino acid)  
10 positions, frequently over a window of at least 24-48 nucleotide (8-16 amino  
acid) positions, wherein the percentage of sequence identity is calculated by  
comparing the reference sequence to the sequence which may include deletions  
or additions which total 20 percent or less of the reference sequence over the  
25 comparison window. The reference sequence may be a subset of a larger  
15 sequence.

30 As used herein, the twenty conventional amino acids and their  
abbreviations follow conventional usage. See *Immunology - A Synthesis* (2<sup>nd</sup>  
Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass.  
20 (1991)), which is incorporated herein by reference. Stereoisomers (e.g., D-  
amino acids) of the twenty conventional amino acids, unnatural amino acids  
35 such as  $\alpha$ -,  $\alpha$ -disubstituted amino acids, N-alkyl amino acids, lactic acid, and  
other unconventional amino acids may also be suitable components for  
polypeptides of the present invention. Examples of unconventional amino acids  
40 25 include: 4-hydroxyproline,  $\gamma$ -carboxyglutamate,  $\epsilon$ -N,N,N-trimethyllysine,  $\epsilon$ -N-  
acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-  
methylhistidine, 5-hydroxylysine,  $\sigma$ -N-methylarginine, and other similar amino  
45 acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used  
herein, the lefthand direction is the amino terminal direction and the righthand  
30 direction is the carboxy-terminal direction, in accordance with standard usage  
and convention.

5 Similarly, unless specified otherwise, the lefthand end of single-stranded polynucleotide sequences is the 5' end; the lefthand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 10 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the 5 RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same 15 sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

10 As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at 25 15 least 95 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is 30 glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and 35 tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is 40 25 cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

45 30 As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being 50 encompassed by the present invention, providing that the variations in the

5 amino acid sequence maintain at least 75%, more preferably at least 80%, 90%,  
95%, and most preferably 99%. In particular, conservative amino acid  
10 replacements are contemplated. Conservative replacements are those that take  
place within a family of amino acids that are related in their side chains.  
5 Genetically encoded amino acids are generally divided into families: (1)  
acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-  
15 polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine,  
tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine,  
serine, threonine, tyrosine. More preferred families are: serine and threonine are  
10 aliphatic-hydroxy family; asparagine and glutamine are an amide-containing  
family; alanine, valine, leucine and isoleucine are an aliphatic family; and  
20 phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it  
is reasonable to expect that an isolated replacement of a leucine with an  
isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or  
25 a similar replacement of an amino acid with a structurally related amino acid  
will not have a major effect on the binding or properties of the resulting  
molecule, especially if the replacement does not involve an amino acid within a  
30 framework site. Whether an amino acid change results in a functional peptide  
can readily be determined by assaying the specific activity of the polypeptide  
20 derivative. Assays are described in detail herein. Fragments or analogs of  
antibodies or immunoglobulin molecules can be readily prepared by those of  
35 ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or  
analogues occur near boundaries of functional domains. Structural and functional  
domains can be identified by comparison of the nucleotide and/or amino acid  
40 sequence data to public or proprietary sequence databases. Preferably,  
computerized comparison methods are used to identify sequence motifs or  
predicted protein conformation domains that occur in other proteins of known  
45 structure and/or function. Methods to identify protein sequences that fold into a  
known three-dimensional structure are known. Bowie et al. *Science* 253:164  
30 (1991). Thus, the foregoing examples demonstrate that those of skill in the art  
can recognize sequence motifs and structural conformations that may be used to  
define structural and functional domains in accordance with the invention.



5 Preferred amino acid substitutions are those which: (1) reduce  
susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter  
10 binding affinity for forming protein complexes, (4) alter binding affinities, and  
5 (4) confer or modify other physicochemical or functional properties of such  
analogs. Analogs can include various muteins of a sequence other than the  
naturally-occurring peptide sequence. For example, single or multiple amino  
15 acid substitutions (preferably conservative amino acid substitutions) may be  
made in the naturally-occurring sequence (preferably in the portion of the  
polypeptide outside the domain(s) forming intermolecular contacts. A  
10 conservative amino acid substitution should not substantially change the  
structural characteristics of the parent sequence (e.g., a replacement amino acid  
should not tend to break a helix that occurs in the parent sequence, or disrupt  
other types of secondary structure that characterizes the parent sequence).  
25 Examples of art-recognized polypeptide secondary and tertiary structures are  
described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W.  
H. Freeman and Company, New York (1984)); *Introduction to Protein  
Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y.  
30 (1991)); and Thornton et al. *Nature* 354:105 (1991), which are each  
20 incorporated herein by reference.

35 The term "polypeptide fragment" as used herein refers to a polypeptide  
that has an amino-terminal and/or carboxy-terminal deletion, but where the  
remaining amino acid sequence is identical to the corresponding positions in the  
25 naturally-occurring sequence deduced, for example, from a full-length cDNA  
sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long,  
preferably at least 14 amino acids long, more preferably at least 20 amino acids  
long, usually at least 50 amino acids long, and even more preferably at least 70  
45 amino acids long. The term "analog" as used herein refers to polypeptides  
30 which are comprised of a segment of at least 25 amino acids that has substantial  
identity to a portion of a deduced amino acid sequence and which has at least  
one of the following properties: (1) specific binding to CTLA-4, under suitable  
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5 binding conditions, (2) ability to block CTLA-4 binding with its receptors, or  
(3) ability to inhibit CTLA-4 expressing cell growth *in vitro* or *in vivo*.  
Typically, polypeptide analogs comprise a conservative amino acid substitution  
10 (or addition or deletion) with respect to the naturally-occurring sequence.  
5 Analog typically are at least 20 amino acids long, preferably at least 50 amino  
acids long or longer, and can often be as long as a full-length naturally-  
occurring polypeptide.

15  
Peptide analogs are commonly used in the pharmaceutical industry as  
10 non-peptide drus with properties analogous to those of the template peptide.  
These types of non-peptide compound are termed "peptide mimetics" or  
20 "peptidomimetics". Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and  
Freidinger *TINS* p.392 (1985); and Evans et al. *J. Med. Chem.* 30:1229 (1987),  
which are incorporated herein by reference. Such compounds are often  
25 developed with the aid of computerized molecular modeling. Peptide mimetics  
that are structurally similar to therapeutically useful peptides may be used to  
produce an equivalent therapeutic or prophylactic effect. Generally,  
30 peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a  
polypeptide that has a biochemical property or pharmacological activity), such  
20 as human antibody, but have one or more peptide linkages optionally replaced  
by a linkage selected from the group consisting of: --CH<sub>2</sub>NH--, --CH<sub>2</sub>S--, --  
35 CH<sub>2</sub>-CH<sub>2</sub>--, --CH=CH--(cis and trans), --COCH<sub>2</sub>--, --CH(OH)CH<sub>2</sub>--, and --  
CH<sub>2</sub>SO--, by methods well known in the art. Systematic substitution of one or  
more amino acids of a consensus sequence with a D-amino acid of the same  
40 type (e.g., D-lysine in place of L-lysine) may be used to generate more stable  
peptides. In addition, constrained peptides comprising a consensus sequence or  
a substantially identical consensus sequence variation may be generated by  
45 methods known in the art (Rizo and Gierasch *Ann. Rev. Biochem.* 61:387  
(1992), incorporated herein by reference); for example, by adding internal  
30 cysteine residues capable of forming intramolecular disulfide bridges which  
cyclize the peptide.

5 "Antibody" or "antibody peptide(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. Binding fragments are produced by recombinant DNA techniques, or  
10 by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', F(ab')<sub>2</sub>, Fv, and single-chain antibodies. An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. An antibody substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at least about 20%, 40%, 60% or 80%, and more  
15 usually greater than about 85% (as measured in an *in vitro* competitive binding assay).  
20

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually  
25 consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is  $\leq 1 \mu\text{M}$ , preferably  
30  $\leq 100 \text{ nM}$  and most preferably  $\leq 10 \text{ nM}$ .  
20

The term "agent" is used herein to denote a chemical compound, a  
35 mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

40 25 As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations,  
45 30 the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the  
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5 following: radioisotopes or radionuclides (e.g.,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{99}\text{Tc}$ ,  
10  $^{111}\text{In}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ), fluorescent labels (e.g., FITC, rhodamine, lanthanide  
phosphors), enzymatic labels (e.g., horseradish peroxidase,  $\beta$ -galactosidase,  
15 luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups,  
predetermined polypeptide epitopes recognized by a secondary reporter (e.g.,  
leucine zipper pair sequences, binding sites for secondary antibodies, metal  
binding domains, epitope tags). In some embodiments, labels are attached by  
spacer arms of various lengths to reduce potential steric hindrance.

10 The term "pharmaceutical agent or drug" as used herein refers to a  
20 chemical compound or composition capable of inducing a desired therapeutic  
effect when properly administered to a patient. Other chemistry terms herein  
are used according to conventional usage in the art, as exemplified by *The*  
25 *McGraw-Hill Dictionary of Chemical Terms* (Parker, S., Ed., McGraw-Hill, San  
15 Francisco (1985)), incorporated herein by reference).

30 The term "antineoplastic agent" is used herein to refer to agents that  
have the functional property of inhibiting a development or progression of a  
neoplasm in a human, particularly a malignant (cancerous) lesion, such as a  
20 carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is  
frequently a property of antineoplastic agents.

35 As used herein, "substantially pure" means an object species is the  
predominant species present (i.e., on a molar basis it is more abundant than any  
40 other individual species in the composition), and preferably a substantially  
purified fraction is a composition wherein the object species comprises at least  
about 50 percent (on a molar basis) of all macromolecular species present.  
45 Generally, a substantially pure composition will comprise more than about 80  
percent of all macromolecular species present in the composition, more  
30 preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the  
object species is purified to essential homogeneity (contaminant species cannot

be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

The term patient includes human and veterinary subjects.

#### Antibody Structure

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions

of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J. Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "'Diabodies': small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)). Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

#### Human Antibodies and Humanization of Antibodies

Human antibodies avoid certain of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, it has been postulated that one can develop humanized antibodies or generate fully human antibodies through the introduction of human antibody function into a rodent so that the rodent would produce antibodies having fully human sequences.

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Human Antibodies

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The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

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An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Mabs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, and cancer, which require repeated antibody administrations.

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One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance

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5 to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human Mabs with the desired specificity could be readily produced and  
10 selected.

5 This general strategy was demonstrated in connection with our generation of the first XenoMouse™ strains as published in 1994. See Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb and  
15 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes.

20 This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human Mabs. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might  
25 recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XenoMouse™ mice. See Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998), and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by  
35 reference.

40 Such approach is further discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/610,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30,  
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5 1992, filed 08/031,801, filed March 15, 1993, 08/112,848, filed August 27,  
1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995,  
08/430, 938, April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed  
10 June 5, 1995, 08/463,191, filed June 5, 1995, 08/462,837, filed June 5, 1995,  
5 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859,  
filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2,  
1996, and 08/759,620, filed December 3, 1996. *See also* Mendez et al. *Nature*  
15 *Genetics* 15:146-156 (1997) and Green and Jakobovits *J. Exp. Med.* 188:483-  
495 (1998). *See also* European Patent No., EP 0 463 151 B1, grant published  
10 June 12, 1996, International Patent Application No., WO 94/02602, published  
February 3, 1994, International Patent Application No., WO 96/34096,  
20 published October 31, 1996, and WO 98/24893, published June 11, 1998. The  
disclosures of each of the above-cited patents, applications, and references are  
hereby incorporated by reference in their entirety.

25 In an alternative approach, others, including GenPharm International,  
Inc., have utilized a "minilocus" approach. In the minilocus approach, an  
exogenous Ig locus is mimicked through the inclusion of pieces (individual  
30 genes) from the Ig locus. Thus, one or more  $V_H$  genes, one or more  $D_H$  genes,  
one or more  $J_H$  genes, a mu constant region, and a second constant region  
20 (preferably a gamma constant region) are formed into a construct for insertion  
into an animal. This approach is described in U.S. Patent No. 5,545,807 to  
35 Surani et al. and U.S. Patent Nos. 5,545,806, 5,625,825, 5,625,126, 5,633,425,  
5,661,016, 5,770,429, 5,789,650, and 5,814,318 each to Lonberg and Kay, U.S.  
Patent No. 5,591,669 to Krimpenfort and Berns, U.S. Patent Nos. 5,612,205,  
40 5,721,367, 5,789,215 to Berns et al., and U.S. Patent No. 5,643,763 to Choi and  
Dunn, and GenPharm International U.S. Patent Application Serial Nos.  
07/574,748, filed August 29, 1990, 07/575,962, filed August 31, 1990,  
07/810,279, filed December 17, 1991, 07/853,408, filed March 18, 1992,  
45 07/904,068, filed June 23, 1992, 07/990,860, filed December 16, 1992,  
30 08/053,131, filed April 26, 1993, 08/096,762, filed July 22, 1993, 08/155,301,  
filed November 18, 1993, 08/161,739, filed December 3, 1993, 08/165,699,  
filed December 10, 1993, 08/209,741, filed March 9, 1994, the disclosures of

5 which are hereby incorporated by reference. *See also* European Patent No. 0  
546 073 B1, International Patent Application Nos. WO 92/03918, WO  
92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO  
10 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884, the disclosures of  
5 which are hereby incorporated by reference in their entirety. *See further* Taylor  
et al., 1992, Chen et al., 1993, Tuailon et al., 1993, Choi et al., 1993, Lonberg  
et al., (1994), Taylor et al., (1994), and Tuailon et al., (1995), Fishwild et al.,  
15 (1996), the disclosures of which are hereby incorporated by reference in their  
entirety.

10 The inventors of Surani et al., cited above and assigned to the Medical  
Research Counsel (the "MRC"), produced a transgenic mouse possessing an Ig  
locus through use of the minilocus approach. The inventors on the GenPharm  
International work, cited above, Lonberg and Kay, following the lead of the  
present inventors, proposed inactivation of the endogenous mouse Ig locus  
25 coupled with substantial duplication of the Surani et al. work.

An advantage of the minilocus approach is the rapidity with which  
constructs including portions of the Ig locus can be generated and introduced  
30 into animals. Commensurately, however, a significant disadvantage of the  
minilocus approach is that, in theory, insufficient diversity is introduced through  
20 the inclusion of small numbers of V, D, and J genes. Indeed, the published  
work appears to support this concern. B-cell development and antibody  
production of animals produced through use of the minilocus approach appear  
35 stunted. Therefore, research surrounding the present invention has consistently  
been directed towards the introduction of large portions of the Ig locus in order  
25 to achieve greater diversity and in an effort to reconstitute the immune  
repertoire of the animals.

Human anti-mouse antibody (HAMA) responses have led the industry to  
prepare chimeric or otherwise humanized antibodies. While chimeric  
45 antibodies have a human constant region and a murine variable region, it is  
30 expected that certain human anti-chimeric antibody (HACA) responses will be  
observed, particularly in chronic or multi-dose utilizations of the antibody.

Thus, it would be desirable to provide fully human antibodies against CTLA-4 in order to vitiate concerns and/or effects of HAMA or HACA response.

Humanization and Display Technologies

As was discussed above in connection with human antibody generation, there are advantages to producing antibodies with reduced immunogenicity. To a degree, this can be accomplished in connection with techniques of humanization and display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art. See e.g., Winter and Harris *Immunol Today* 14:43-46 (1993) and Wright et al. *Crit. Reviews in Immunol.* 12:125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence (see WO 92/02190 and U.S. Patent Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,792, 5,714,350, and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu et al. *P.N.A.S.* 84:3439 (1987) and *J.Immunol.*139:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683,195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant regions genes may be found in Kabat et al. (1991) *Sequences of Proteins of Immunological Interest*, N.I.H. publication no. 91-3242. Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Preferred isotypes are IgG1, IgG2, IgG3 and IgG4. Particularly preferred isotypes for antibodies of the invention are IgG2 and IgG4. Either of the human light chain constant regions, kappa or

5 lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

10 Antibody fragments, such as Fv, F(ab')<sub>2</sub> and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage.  
5 Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')<sub>2</sub> fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a  
15 translational stop codon to yield the truncated molecule.

In one approach, consensus sequences encoding the heavy and light  
10 chain J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V  
20 region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

25 Expression vectors include plasmids, retroviruses, cosmids, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be  
30 easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within  
35 the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral  
40 LTRs, e.g. SV-40 early promoter, (Okayama et al. *Mol. Cell. Bio.* 3:280 (1983)), Rous sarcoma virus LTR (Gorman et al. *P.N.A.S.* 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl et al. *Cell* 41:885 (1985)); native Ig promoters, etc.

45 Further, human antibodies or antibodies from other species can be  
30 generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to  
50

5 additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright and Harris, *supra.*, Hanes and Plutchau *PNAS USA* 94:4937-4942 (1997) (ribosomal display), Parmley and Smith *Gene* 73:305-318 (1988) (phage display), Scott *TIBS* 17:241-245 (1992), Cwirla et al. *PNAS USA* 10 87:6378-6382 (1990), Russel et al. *Nucl. Acids Research* 21:1081-1085 (1993), Hoganboom et al. *Immunol. Reviews* 130:43-68 (1992), Chiswell and McCafferty *TIBTECH* 10:80-84 (1992), and U.S. Patent No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

15 Using these techniques, antibodies can be generated to CTLA-4 expressing cells, CTLA-4 itself, forms of CTLA-4, epitopes or peptides thereof, and expression libraries thereto (*see e.g.* U.S. Patent No. 5,703,057) which can thereafter be screened as described above for the activities described above.

#### 25 Additional Criteria for Antibody Therapeutics

30 As will be appreciated, it is generally not desirable to kill CTLA-4 expressing cells. Rather, one generally desires to simply inhibit CTLA-4 binding with its ligands to mitigate T cell down regulation. One of the major mechanisms through which antibodies kill cells is through fixation of complement and participation in CDC. The constant region of an antibody plays an important role in connection with an antibody's ability to fix complement and participate in CDC. Thus, generally one selects the isotype of an antibody to either provide the ability of complement fixation, or not. In the case of the present invention, generally, as mentioned above, it is generally not preferred to utilize an antibody that kills the cells. There are a number of isotypes of antibodies that are capable of complement fixation and CDC, including, without limitation, the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, and human IgG3. Those isotypes that do not include, without limitation, human IgG2 and human IgG4.

50 It will be appreciated that antibodies that are generated need not initially possess a particular desired isotype but, rather, the antibody as generated can

5 possess any isotype and the antibody can be isotype switched thereafter using  
conventional techniques that are well known in the art. Such techniques include  
the use of direct recombinant techniques (*see e.g.*, U.S. Patent No. 4,816,397),  
10 cell-cell fusion techniques (*see e.g.*, U.S. Patent Application No. 08/730,639,  
5 filed October 11, 1996), among others.

In the cell-cell fusion technique, a myeloma or other cell line is prepared  
that possesses a heavy chain with any desired isotype and another myeloma or  
15 other cell line is prepared that possesses the light chain. Such cells can,  
thereafter, be fused and a cell line expressing an intact antibody can be isolated.

10 By way of example, the majority of the CTLA-4 antibodies discussed  
herein are human anti-CTLA-4 IgG2 antibody. Since such antibodies possess  
desired binding to the CTLA-4 molecule, any one of such antibodies can be  
readily isotype switched to generate a human IgG4 isotype, for example, while  
20 still possessing the same variable region (which defines the antibody's  
25 specificity and some of its affinity).

Accordingly, as antibody candidates are generated that meet desired  
"structural" attributes as discussed above, they can generally be provided with  
at least certain additional "functional" attributes that are desired through isotype  
30 switching.

#### 20 Design and Generation of Other Therapeutics

35 In accordance with the present invention and based on the activity of the  
antibodies that are produced and characterized herein with respect to CTLA-4,  
25 the design of other therapeutic modalities including other antibodies, other  
antagonists, or chemical moieties other than antibodies is facilitated. Such  
modalities include, without limitation, antibodies having similar binding activity  
or functionality, advanced antibody therapeutics, such as bispecific antibodies,  
45 immunotoxins, and radiolabeled therapeutics, generation of peptide  
30 therapeutics, gene therapies, particularly intrabodies, antisense therapeutics, and  
small molecules. Furthermore, as discussed above, the effector function of the

antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses.

In connection with the generation of advanced antibody therapeutics, where complement fixation is a desirable attribute, it may be possible to sidestep the dependence on complement for cell killing through the use of bispecifics, immunotoxins, or radiolabels, for example.

In connection with bispecific antibodies, bispecific antibodies can be generated that comprise (i) two antibodies one with a specificity to CTLA-4 and another to a second molecule that are conjugated together, (ii) a single antibody that has one chain specific to CTLA-4 and a second chain specific to a second molecule, or (iii) a single chain antibody that has specificity to CTLA-4 and the other molecule. Such bispecific antibodies can be generated using techniques that are well known for example, in connection with (i) and (ii) see e.g., Fanger et al. *Immunol Methods* 4:72-81 (1994) and Wright and Harris, *supra.* and in connection with (iii) see e.g., Traunecker et al. *Int. J. Cancer (Suppl.)* 7:51-52 (1992).

In addition, "Kappabodies" (Ill et al. "Design and construction of a hybrid immunoglobulin domain with properties of both heavy and light chain variable regions" *Protein Eng* 10:949-57 (1997)), "Minibodies" (Martin et al. "The affinity-selection of a minibody polypeptide inhibitor of human interleukin-6" *EMBO J* 13:5303-9 (1994)), "Diabodies" (Holliger et al. "'Diabodies': small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)), or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)) may also be prepared.

In connection with immunotoxins, antibodies can be modified to act as immunotoxins utilizing techniques that are well known in the art. See e.g., Vitetta *Immunol Today* 14:252 (1993). See also U.S. Patent No. 5,194,594. In connection with the preparation of radiolabeled antibodies, such modified antibodies can also be readily prepared utilizing techniques that are well known

5 in the art. See e.g., Junghans et al. in *Cancer Chemotherapy and Biotherapy*  
655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)). See  
also U.S. Patent Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (RE 35,500),  
10 5,648,471, and 5,697,902. Each of immunotoxins and radiolabeled molecules  
5 would be likely to kill cells expressing CTLA-4, and particularly those cells in  
which the antibodies of the invention are effective.

15 In connection with the generation of therapeutic peptides, through the  
utilization of structural information related to CTLA-4 and antibodies thereto,  
such as the antibodies of the invention (as discussed below in connection with  
10 small molecules) or screening of peptide libraries, therapeutic peptides can be  
generated that are directed against CTLA-4. Design and screening of peptide  
20 therapeutics is discussed in connection with Houghten et al. *Biotechniques*  
13:412-421 (1992), Houghten *PNAS USA* 82:5131-5135 (1985), Pinalla et al.  
*Biotechniques* 13:901-905 (1992), Blake and Litzi-Davis *BioConjugate Chem.*  
25 3:510-513 (1992). Immunotoxins and radiolabeled molecules can also be  
prepared, and in a similar manner, in connection with peptidic moieties as  
discussed above in connection with antibodies.

30 Important information related to the binding of an antibody to an antigen  
can be gleaned through phage display experimentation. Such experiments are  
20 generally accomplished through panning a phage library expressing random  
peptides for binding with the antibodies of the invention to determine if peptides  
35 can be isolated that bind. If successful, certain epitope information can be  
gleaned from the peptides that bind.

40 In general, phage libraries expressing random peptides can be purchased  
25 from New England Biolabs (7-mer and 12-mer libraries, Ph.D.-7 Peptide 7-mer  
Library Kit and Ph.D.-12 Peptide 12-mer Library Kit, respectively) based on a  
bacteriophage M13 system. The 7-mer library represents a diversity of  
approximately  $2.0 \times 10^9$  independent clones, which represents most, if not all,  
45 of the  $20^7 = 1.28 \times 10^9$  possible 7-mer sequences. The 12-mer library contains  
30 approximately  $1.9 \times 10^9$  independent clones and represents only a very small  
sampling of the potential sequence space of  $20^{12} = 4.1 \times 10^{15}$  12-mer sequences.  
Each of 7-mer and 12-mer libraries are panned or screened in accordance with



5 the manufacturer's recommendations in which plates were coated with an  
antibody to capture the appropriate antibody (a goat anti-human IgG Fc for an  
10 IgG antibody for example) followed by washing. Bound phage are eluted with  
0.2 M glycine-HCl, pH 2.2. After 3 rounds of selection/amplification at  
5 constant stringency (0.5% Tween), through use of DNA sequencing, one can  
characterize clones from the libraries that are reactive with one or more of the  
15 antibodies. Reactivity of the peptides can be determined by ELISA. For an  
additional discussion of epitope analysis of peptides *see also* Scott, J.K. and  
Smith, G.P. *Science* 249:386-390 (1990); Cwirla et al. *PNAS USA* 87:6378-  
10 6382 (1990); Felici et al. *J. Mol. Biol.* 222:301-310 (1991), and Kuwabara et al.  
20 *Nature Biotechnology* 15:74-78 (1997).

The design of gene and/or antisense therapeutics through conventional  
techniques is also facilitated through the present invention. Such modalities can  
be utilized for modulating the function of CTLA-4. In connection therewith the  
25 antibodies of the present invention facilitate design and use of functional assays  
related thereto. A design and strategy for antisense therapeutics is discussed in  
15 detail in International Patent Application No. WO 94/29444. Design and  
strategies for gene therapy are well known. However, in particular, the use of  
30 gene therapeutic techniques involving intrabodies could prove to be particularly  
advantageous. *See e.g.*, Chen et al. *Human Gene Therapy* 5:595-601 (1994) and  
20 Marasco *Gene Therapy* 4:11-15 (1997). General design of and considerations  
related to gene therapeutics is also discussed in International Patent Application  
35 No. WO 97/38137. Genetic materials encoding an antibody of the invention  
(such as the 4.1.1, 4.8.1, or 6.1.1, or others) may be included in a suitable  
40 25 expression system (whether viral, attenuated viral, non-viral, naked, or  
otherwise) and administered to a host for *in vivo* generation of the antibody in  
the host.

Small molecule therapeutics can also be envisioned in accordance with  
45 the present invention. Drugs can be designed to modulate the activity of CTLA-  
30 4 based upon the present invention. Knowledge gleaned from the structure of  
the CTLA-4 molecule and its interactions with other molecules in accordance  
with the present invention, such as the antibodies of the invention, CD28, B7,  
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5 B7-1, B7-2, and others can be utilized to rationally design additional therapeutic modalities. In this regard, rational drug design techniques such as X-ray crystallography, computer-aided (or assisted) molecular modeling (CAMP),  
10 quantitative or qualitative structure-activity relationship (QSAR), and similar technologies can be utilized to focus drug discovery efforts. Rational design allows prediction of protein or synthetic structures which can interact with the molecule or specific forms thereof which can be used to modify or modulate the  
15 activity of CTLA-4. Such structures can be synthesized chemically or expressed in biological systems. This approach has been reviewed in Capsey et al. *Genetically Engineered Human Therapeutic Drugs* (Stockton Press, NY  
20 (1988)). Indeed, the rational design of molecules (either peptides, peptidomimetics, small molecules, or the like) based upon known, or delineated, structure-activity relationships with other molecules (such as antibodies in accordance with the invention) has become generally routine. See, e.g., Fry et  
25 al. "Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor" *Proc Natl Acad Sci U S A* 95:12022-7 (1998); Hoffman et al. "A model of Cdc25 phosphatase catalytic domain and Cdk-interaction surface based on the presence of a rhodanese  
30 homology domain" *J Mol Biol* 282:195-208 (1998); Ginalski et al. "Modelling of active forms of protein kinases: p38--a case study" *Acta Biochim Pol* 44:557-64 (1997); Jouko et al. "Identification of csk tyrosine phosphorylation sites and a tyrosine residue important for kinase domain structure" *Biochem J* 322:927-35  
35 (1997); Singh et al. "Structure-based design of a potent, selective, and irreversible inhibitor of the catalytic domain of the erbB receptor subfamily of protein tyrosine kinases" *J Med Chem* 40:1130-5 (1997); Mandel et al.  
40 "ABGEN: a knowledge-based automated approach for antibody structure modeling" *Nat Biotechnol* 14:323-8 (1996); Monfardini et al. "Rational design, analysis, and potential utility of GM-CSF antagonists" *Proc Assoc Am Physicians* 108:420-31 (1996); Furet et al. "Modelling study of protein kinase  
45 inhibitors: binding mode of staurosporine and origin of the selectivity of CGP 52411" *J Comput Aided Mol Des* 9:465-72 (1995).

Further, combinatorial libraries can be designed and synthesized and used in screening programs, such as high throughput screening efforts.

#### Therapeutic Administration and Formulations

It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15<sup>th</sup> ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin<sup>TM</sup>), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. "Compendium of excipients for parenteral formulations" *PDA J Pharm Sci Technol.* 52:238-311 (1998) and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

#### Preparation of Antibodies

Antibodies in accordance with the invention are preferably prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine, antibodies. Such mice, then, are capable of

5 producing human immunoglobulin molecules and antibodies and are deficient  
in the production of murine immunoglobulin molecules and antibodies.  
Technologies utilized for achieving the same are disclosed in the patents,  
10 applications, and references disclosed in the Background, herein. In particular,  
5 however, a preferred embodiment of transgenic production of mice and  
antibodies therefrom is disclosed in U.S. Patent Application Serial No.  
08/759,620, filed December 3, 1996, the disclosure of which is hereby  
15 incorporated by reference. *See also* Mendez et al. *Nature Genetics* 15:146-156  
(1997), the disclosure of which is hereby incorporated by reference.

10 Through use of such technology, we have produced fully human  
monoclonal antibodies to a variety of antigens. Essentially, we immunize  
XenoMouse™ lines of mice with an antigen of interest, recover lymphatic cells  
(such as B-cells) from the mice that express antibodies, fuse such recovered  
20 cells with a myeloid-type cell line to prepare immortal hybridoma cell lines, and  
25 such hybridoma cell lines are screened and selected to identify hybridoma cell  
lines that produce antibodies specific to the antigen of interest. We utilized  
these techniques in accordance with the present invention for the preparation of  
antibodies specific to CTLA-4. Herein, we describe the production of multiple  
30 hybridoma cell lines that produce antibodies specific to CTLA-4. Further, we  
20 provide a characterization of the antibodies produced by such cell lines,  
including nucleotide and amino acid sequence analyses of the heavy and light  
chains of such antibodies.

The antibodies derived from hybridoma cell lines discussed herein are  
designated 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1,  
40 25 11.7.1, 12.3.1.1, and 12.9.1.1. Each of the antibodies produced by the  
aforementioned cell lines are either fully human IgG2 or IgG4 heavy chains  
with human kappa light chains. In general, antibodies in accordance with the  
invention possess very high affinities, typically possessing Kd's of from about  
45  $10^{-9}$  through about  $10^{-11}$  M, when measured by either solid phase or solution  
30 phase.

As will be appreciated, antibodies in accordance with the present  
invention can be expressed in cell lines other than hybridoma cell lines.

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Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO<sub>0</sub>, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The expression methods are selected by determining which system generates the highest expression levels and produce antibodies with constitutive CTLA-4 binding properties.

Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase and DHFR gene expression systems are common approaches for enhancing expression under certain

5 conditions. High expressing cell clones can be identified using conventional techniques, such as limited dilution cloning and Microdrop technology. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 5 89303964.4.

15 Antibodies of the invention can also be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in 10 mammals, antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. *See, e.g.*, U.S. Patent Nos. 5,827,690, 5,756,687, 20 5,750,172, and 5,741,957.

25 Antibodies in accordance with the present invention have been analyzed structurally and functionally. In connection with the structures of the antibodies, amino acid sequences of the heavy and kappa light chains have been 15 predicted based on cDNA sequences obtained through RT-PCR of the hybridomas. *See* Examples 3 and 4 and Figures 1-8. N-terminal sequencing of the antibodies was also conducted in confirmation of the results discussed in 30 Examples 3 and 4. *See* Example 5 and Figure 9. Kinetic analyses of the antibodies were conducted to determine affinities. *See* Example 2. Antibodies in accordance with the invention (and particularly the 4.1.1, 4.8.1, and 6.1.1 35 antibodies of the invention) have high affinities ( $4.1.1:1.63 \times 10^{10}$  1/M;  $4.8.1:3.54 \times 10^{10}$  1/M; and  $6.1.1:7.2 \times 10^9$  1/M). Further, antibodies were analyzed by isoelectric focusing (IEF), reducing gel electrophoresis (SDS- 40 25 PAGE), size exclusion chromatography, liquid chromatography/mass spectroscopy, and mass spectroscopy and antibody production by the hybridomas was assessed. *See* Example 6 and Figure 10.

45 In connection with functional analysis of antibodies in accordance with the present invention, such antibodies proved to be potent inhibitors of CTLA-4 and its binding to its ligands of the B7 family of molecules. For example, 30 antibodies in accordance with the present invention were demonstrated to block CTLA-4 binding to either B7-1 or B7-2. *See* Example 7. Indeed, many of the 50

antibodies in accordance with the invention possess nanomolar and subnanomolar  $IC_{50}$ s with respect to inhibiting CTLA-4 binding to B7-1 and B7-2. Further, antibodies of the invention possess excellent selectivity for CTLA-4 as compared to CD28, CD44, B7-2, or hIgG1. See Example 8. Selectivity is a ratio that reflects the degree of preferential binding of a molecule with a first agent as compared to the molecules binding with a second, and optionally other molecules. Herein, selectivity refers to the degree of preferential binding of an antibody of the invention to CTLA-4 as compared to the antibody's binding to other molecules such as CD28, CD44, B7-2, or hIgG1. Selectivity values of antibodies of the invention greater than 500:1 are common. Antibodies of the invention have also been demonstrated to induce or enhance expression of certain cytokines (such as IL-2 and IFN- $\gamma$ ) by cultured T cells in a T cell blast model. See Examples 9 and 10 and Figures 12-17. Further, it is expected that antibodies of the invention will inhibit the growth of tumors in appropriate *in vivo* tumor models. The design of which models are discussed in Example 11 and 12.

The results demonstrated in accordance with the present invention indicate that antibodies of the present invention possess certain qualities that may make the present antibodies more efficacious than current therapeutic antibodies against CTLA-4.

In particular, the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention possess highly desirable properties. Their structural characteristics, functions, or activities provide criteria that facilitate the design or selection of additional antibodies or other molecules as discussed above. Such criteria include one or more of the following:

Ability to compete for binding to CTLA-4 with one or more of the antibodies of the invention;

Similar binding specificity to CTLA-4 as one or more of the antibodies of the invention;

A binding affinity for CTLA-4 of about  $10^{-9}$  M or greater and preferably of about  $10^{-10}$  M or greater;

Does not cross react with lower mammalian CTLA-4, including, preferably, mouse, rat, or rabbit and preferably mouse or rat CTLA-4;

Cross reacts with primate CTLA-4, including, preferably, cynomolgous and rhesus CTLA-4;

A selectivity for CTLA-4 over CD28, B7-2, CD44, or hlgG1 of at least about 100:1 or greater and preferably of about 300, 400, or 500:1 or greater;

An  $IC_{50}$  in blocking CTLA-4 binding to B7-2 of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.38 nM or lower;

An  $IC_{50}$  in blocking CTLA-4 binding to B7-1 of about of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.50 nM or lower;

An enhancement of cytokine production in one or more *in vitro* assays, for example:

An enhancement of IL-2 production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, 1500, 2000, 3000, or 3846 pg/ml or greater;

An enhancement of IFN- $\gamma$  production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, or 1233 pg/ml or greater; or

An enhancement of IL-2 production in a hPBMC or whole blood superantigen assay of about 500 pg/ml or greater and preferably 750, 1000, 1200, or 1511 pg/ml or greater. Expressed another way, it is desirable that IL-2 production is enhanced by about 30, 35, 40, 45, 50 percent or more relative to control in the assay.

It is expected that antibodies (or molecules designed or synthesized therefrom) having one or more of these properties will possess similar efficacy to the antibodies described in the present invention.

The desirable functional properties discussed above can often result from binding to and inhibition of CTLA4 by a molecule (i.e., antibody, antibody fragment, peptide, or small molecule) in a similar manner as an antibody of the invention (i.e., binding to the same or similar epitope of the CTLA4 molecule).



5 The molecule may either be administered directly (i.e., direct administration to a patient of such molecules). Or, alternatively, the molecule may be  
10 "administered" indirectly (i.e., a peptide or the like that produces an immune response in a patient (similar to a vaccine) wherein the immune response  
5 includes the generation of antibodies that bind to the same or similar epitope or an antibody or fragment that is produced *in situ* after administration of genetic materials that encode such antibodies or fragments thereof which bind to the  
15 same or similar epitope). Thus, it will be appreciated that the epitope on CTLA4 to which antibodies of the invention bind to can be useful in connection  
10 with the preparation and/or design of therapeutics in accordance with the invention. In drug design, negative information is often useful as well (i.e., the fact that an antibody which binds to CTLA4 does not appear to bind to an epitope that acts as an inhibitor of CTLA4 is useful). Thus, the epitope to  
20 which antibodies of the invention bind that do not lead to the desired functionality can also be very useful. Accordingly, also contemplated in accordance with the present invention are molecules (and particularly antibodies) that bind to the same or similar epitopes as antibodies of the  
25 invention.

30 In addition to the fact that antibodies of the invention and the epitopes to which they bind are contemplated in accordance with the invention, we have conducted some preliminary epitope mapping studies of certain antibodies in  
20 accordance with the invention and particularly the 4.1.1 and the 11.2.1 antibodies of the invention.

35 As a first step, we conducted BIAcore competition studies to generate a rough map of binding as between certain antibodies of the invention in  
40 connection with their ability to compete for binding to CTLA4. To this end, CTLA4 was bound to a BIAcore chip and a first antibody, under saturating conditions, was bound thereto and competition of subsequent secondary  
45 antibodies binding to CTLA4 was measured. This technique enabled generation of a rough map in to which families of antibodies can be classified.  
30

Through this process, we determined that the certain antibodies in accordance with the invention could be categorized as falling into the following epitopic categories:

Category	Antibodies	Competition for CTLA4 Binding
A	BO1M*	Freely cross-compete with one another; cross-compete with category B; some cross-competition with category D
	BO2M**	
B	4.1.1	Freely cross-compete with one another; cross-compete with category A, C and D.
	4.13.1	
C	6.1.1	Freely cross-compete with one another; cross-compete with category B and category D
	3.1.1	
	4.8.1	
	11.2.1	
	11.6.1	
	11.7.1	
D	4.14.3	Cross-compete with category C and B; some cross-competition with category A
E	4.9.1	BNI3 blocks 4.9.1 binding to CTLA4 but not the reverse
	BNI3***	

(\*) (\*\*) Available from Biostride.

(\*\*\*) Available from Pharmingen.

As a next step, we endeavored to determine if the antibodies of the invention recognized a linear epitope on CTLA4 under reducing and non-reducing conditions on Western blots. We observed that none of the 4.1.1, 3.1.1, 11.7.1, 11.6.1, or 11.2.1 antibodies of the invention appeared to recognize a reduced form of CTLA4 on Western blot. Accordingly, it appeared likely that the epitope to which each of these antibodies bound was not a linear epitope but more likely was a conformational epitope the structure of which may have been abrogated under reducing conditions.

Therefore, we sought to determine whether we could learn about residues within the CTLA4 molecule that are important for binding of antibodies of the invention. One manner that we utilized was to conduct kinetic assessments of off-rates as between human CTLA4 and two highly conserved primate CTLA4 molecules (cynomolgous and marmoset CTLA4). BIAcore

5 studies demonstrated that the 4.1.1 antibody of the invention bound to human, cynomologous, and marmoset CTLA4 at the same rate. However, with respect to off-rates (affinity), the 4.1.1 antibody had the highest affinity (slowest off-  
10 rate) for human, a faster off-rate with cynomologous, and a much faster off-rate for marmoset. The 11.2.1 antibody of the invention, on the other hand, binds to human, cynomologous, and marmoset CTLA4 at the about the same rate and has about the same relative off-rate for each of the three. This information  
15 further indicates that the 4.1.1 and 11.2.1 antibodies of the invention bind to different epitopes on CTLA4.

10 To further study the epitope to which the category B and C antibodies of the invention bind, we conducted certain site directed mutagenesis studies. Marmoset CTLA4 possesses two important changes at residues 105 and 106 relative to human CTLA4. Such differences are a leucine to methionine change at residue 105 and a glycine to serine change at residue 106. Accordingly, we  
20 mutated cDNA encoding human CTLA4 to encode a mutated CTLA4 having the L105M and G106S changes. The homologue replacement mutant CTLA4 did not effect binding of a B7.2-IgG1 fusion protein. Further, binding with the 11.2.1 antibody of the invention was not effected. However, such molecule was significantly inhibited in its ability to bind with the 4.1.1 antibody of the  
25 invention (similar to marmoset). Next, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a S106G change. Such change resulted in restoration of stable binding between the 4.1.1 antibody and the marmoset CTLA4 mutant. In addition, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a M105L  
30 change. Such change partially restored binding between the 4.1.1 antibody and the mutant CTLA4.

Each of the category B through D antibodies of the invention appear to possess similar functional properties and appear to have the potential to act as  
45 strong anti-CTLA4 therapeutic agents. Further, each of the molecules certain cross-competition in their binding for CTLA4. However, as will be observed from the above discussion, each of the molecules in the different categories appear to bind to separate conformational epitopes on CTLA4.  
50

From the foregoing, it will be appreciated that the epitope information discussed above indicates that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention will likely have certain therapeutic potential in accordance with the present invention. Further, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) will likely have certain additional therapeutic potential in accordance with the present invention. Additionally, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) and that (i) are not reduced in their binding to marmoset CTLA4 (similar to the 11.2.1 antibody) or (ii) are reduced in their binding to marmoset CTLA4 (similar to the 4.1.1 antibody) will likely have certain additional therapeutic potential in accordance with the present invention. Antibodies (or other molecules, as discussed above) that compete with categories A and E may also have certain therapeutic potential.

### EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the present invention.

#### EXAMPLE 1

##### Generation of Anti-CTLA-4-Antibody Producing Hybridomas

Antibodies of the invention were prepared, selected, and assayed in accordance with the present Example.

**Antigen Preparation:** Three distinct immunogens were prepared for immunization of the XenoMouse™ mice: (i) a CTLA-4-IgG fusion protein, (ii) a CTLA-4 peptide, and (iii) 300.19 murine lymphoma cells transfected with

a mutant of CTLA-4 (Y201V) that is constitutively expressed on the cell surface.

(i) CTLA-4-IgG1 Fusion Protein:

Expression Vector Construction:

The cDNA encoding the mature extracellular domain of CTLA-4 was PCR amplified from human thymus cDNA library (Clontech) using primers designed to published sequence (*Eur. J Immunol* 18:1901-1905 (1988)). The fragment was directionally subcloned into pSR5, a Sindbis virus expression plasmid (InVitrogen), between the human oncostatin M signal peptide and human IgG gamma 1 (IgG1) CH1/CH2/CH3 domains. The fusion protein does not contain a hinge domain but contains cysteine 120 in the extracellular domain of CTLA-4 to form a covalent dimer. The resulting vector was called CTLA-4-IgG1/pSR5. The complete CTLA-4-IgG1 cDNA in the vector was sequence confirmed in both strands. The amino acid sequence the CTLA4-Ig protein is shown below. The mature extracellular domain for CD44 was PCR amplified from human lymphocyte library (Clontech) and subcloned into pSinRep5 to generate a control protein with the identical IgG1 tail.

OM-CTLA4-IgG1 Fusion Protein:

MGVLLTORTLLSLVLALLFPSMASMAMHVAQPAVVLAASSRGIASFVC  
EYASPGKATEVRVTVLRLQADSQVTEVCAATYMMGNELTFLDDSICT  
GTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLIGIGNGTQIY  
VIDPEPCPDSLEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE  
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE  
YKCKVSNKALPTIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL  
VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR  
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Underlined: signal peptide

Bold: CTLA4 extracellular domain

5                   The cDNAs for mature extracellular domain of CD28 were PCR  
amplified from human lymphocyte library (Clontech) and then subcloned into  
pCDM8 (*J. Immunol.* 151: 5261-71 (1993)) to produce a human IgG1 fusion  
protein containing both thrombin cleavage and hinge regions. Marmoset,  
Cynomolgous, and Rhesus CTLA4 were cloned from mRNA isolated from  
10 PHA stimulated PBMCs using standard techniques of degenerate PCR.  
Sequencing demonstrated that rhesus and cynomolgous amino acid sequence  
were identical with three differences from mature human CTLA4 extracellular  
domain (S13N, I17T and L105M). Marmoset demonstrated ten amino acid  
differences from the mature human CTLA4 extracellular domain (V21A, V33I,  
25 A41T, A51G, 54I, S71F, Q75K, T88M, L105M and G106S). Site directed  
mutagenesis was used to make single point mutations of all amino acids  
different in marmoset CTLA4 to map amino acids important for interaction of  
the antibodies with human CTLA4-IgG. Mutations of human and marmoset  
CTLA-IgG for epitope mapping were generated by matchmaker site-directed  
30 mutagenesis (Promega). The IgG fusion proteins were produced by transient  
transfection of Cos7 cells and purified using standard Protein A techniques.  
Mutant CTLA4-IgG proteins were evaluated for binding to antibodies by  
immunoblotting and using BIAcore analyses.

#### 40                   25                   Recombinant Protein Expression/Purification:

Recombinant sindbis virus was generated by electroporating (Gibco)  
Baby Hamster Kidney cells with SP6 *in vitro* transcribed CTLA-4-IgG1/pSR5  
45 mRNA and DH-26S helper mRNA as described by InVitrogen. Forty eight  
30 hours later recombinant virus was harvested and titered for optimal protein  
expression in Chinese hamster ovary cells (CHO-K1). CHO-K1 cells were  
cultured in suspension in DMEM/F12 (Gibco) containing 10% heat-inactivated

5 fetal bovine serum (Gibco), non-essential amino acids (Gibco), 4mM glutamine (Gibco), penicillin/streptomycin (Gibco), 10mM Hepes pH 7.5 (Gibco). To produce CTLA-4-IgG, the CHO-K1 cells were resuspended at  $1 \times 10^7$  cells/ml in DMEM/F12 and incubated with sindbis virus for one hour at room temperature.

10 Cells were then diluted to  $1 \times 10^6$ /ml in DMEM/F12 containing 1% fetal bovine serum depleted of bovine IgG using protein A sepharose (Pharmacia), non-essential amino acids, 4mM glutamine, 12.5mM Hepes pH 7.5, and penicillin/streptomycin. Forty eight hours post-infection cells were pelleted and conditioned media was harvested and supplemented with complete protease inhibitor tablets (Boehringer Mannheim), pH adjusted to 7.5, and filtered

15 0.2 $\mu$  (Nalgene). FPLC (Pharmacia) was used to affinity purify the fusion protein using a 5ml protein A HiTrap column (Pharmacia) at a 10ml/min flow rate. The column was washed with 30 bed volumes of PBS and eluted with 0.1M glycine/HCl pH 2.8 at 1ml/min. Fractions (1ml) were immediately

20 neutralized to pH 7.5 with Tris pH 9. The fractions containing CTLA-4-IgG1 were identified by SDS-PAGE and then concentrated using centrplus 50 (Amicon) before applying to sepharose 200 column (Pharmacia) at 1ml/min using PBS as the solvent. Fractions containing CTLA-4-IgG1 were pooled, sterile filtered 0.2 $\mu$  (Millipore), aliquoted and frozen at -80°C. CD44-IgG1 was

25 expressed and purified using the same methods. CD28-IgG was purified from conditioned media from transiently transfected Cos7 cells.

#### Characterization CTLA-4-IgG1:

40 25 The purified CTLA-4-IgG1 migrated as a single band on SDS-PAGE using colloidal coomassie staining (Novex). Under non-reducing conditions CTLA-4-IgG1 was a dimer (100kDa), that reduced to a 50kDa monomer when treated with 50mM DTT. Amino acid sequencing of the purified CTLA-4-IgG1

45 in solution confirmed the N-terminus of CTLA-4 (MHVAQPAVVLAS), and

30 that the oncostatin-M signal peptide was cleaved from the mature fusion protein. The CTLA-4-IgG1 bound to immobilized B7.1-IgG in a concentration dependent manner and the binding was blocked by a hamster-anti-human anti-

50

CTLA-4 antibody (BN13: PharMingen). The sterile CTLA-4-IgG was endotoxin free and quantitated by OD280 using 1.4 as the extinction coefficient. The yield of purified CTLA-4-IgG ranged between 0.5-3mgs/liter of CHO-K1 cells.

(ii) CTLA-4 Peptide:

The following CTLA-4 peptide was prepared as described below:

NH<sub>2</sub>-MHVAQPAVVLAASSRGLASFVCEYASPGKATEVRVTVLRLQADSQVT  
EVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICK  
VELMYPPPYLGI GNGTQIYVIDPEPC-CONH<sub>2</sub>

Abbreviations/Materials:

NMP, N-Methylpyrrolidinone; TFE, 2,2,2-Trifluoroethanol; DCM, Dichloromethane; FMOC, Fluorenyl Methoxycarbonyl. All reagents were supplied by Perkin Elmer, with the following exceptions: TFE, Aldrich Chemical, FMOC-PAL-PEG resin, Perseptive Biosystems. Fmoc-Arg(PMC)-OH, FMOC-Asn(Trt)-OH, FMOC-Asp(tBu)-OH, FMOC-Cys(Trt)-OH, FMOC-Glu(tBu)-OH, FMOC-Gln(Trt)-OH, FMOC-His(Boc)-OH, FMOC-Lys(BOC)-OH, FMOC-Ser(tBu)-OH, FMOC-Thr(tBu)-OH and FMOC-Tyr(tBu)-OH were used for those amino acids requiring side chain protecting groups

Peptide Synthesis:

Peptide synthesis was performed on a Perkin-Elmer 431A, retrofitted with feedback monitoring via UV absorbance at 301nm (Perkin-Elmer Model 759A detector). The peptide sequence was assembled on a FMOC-PAL-PEG resin using conditional double coupling cycles. Forced double couplings were performed at cycles 10,11,18,19,20 and 28 through 33. The resin was washed with a 50% mixture of DCM and TFE at the completion of each acylation cycle,



5 followed by capping of unreacted amino groups with acetic anhydride in NMP. Resin was removed from the reactor after completing cycle 49 and the remainder continued to completion. Peptide cleavage from the resin was  
10 performed using Reagent K (King et al. *International Journal of Protein and Peptide Research* 36:255-266 (1990)) for 6 hours on 415mg of resin affording  
5 186mg crude CTLA-4 peptide.

15 Peptide Characterization:

10 25mg aliquots of the crude CTLA-4 peptide were dissolved in 5ml 6M Guanidine HCl/100mM K<sub>2</sub>PO<sub>3</sub> at pH6.4 and eluted over a Pharmacia Hi Load Superdex 75 16/60 column (16mm x 600mm, 120ml bed volume) with 2M  
20 Guanidine.HCl / 100mM K<sub>2</sub>PO<sub>3</sub> at pH6.4 at 2 ml / min for 180 minutes collecting 5 ml fractions. The fractions were analyzed by loading 1.7µl of  
25 fractions onto a NuPAGE Laemeli gel running with MES running buffer and visualizing via Daichii silver stain protocol. Those fractions exhibiting a molecular weight of 12 KDa, as judged versus molecular weight standards,  
30 were pooled together and stored at 4°C. The combined fractions were analyzed by UV and gel electrophoresis. Amino acid sequencing was performed by absorbing a 100 microliter sample in a ProSorb cartridge (absorbed onto a PVDF membrane) and washing to remove the buffer salts. Sequencing was  
35 performed on an Applied Biosystems 420. The expected N-terminal sequence (M H V A Q P A V V L A) was observed. Immunoblotting demonstrated that the peptide was recognized by the BNI3 anti-human CTLA-4 (PharMingen).  
40 To desalt, an aliquot containing 648µg of material was placed in 3500 Da MWCO dialysis tubing and dialyzed against 0.1% TFA / H<sub>2</sub>O at 4°C for 9 days with stirring. The entire contents of the dialysis bag was lyophilized to a  
45 powder.

(iii) 300.19 cells transfected with CTLA-4 (Y201V)

The full length CTLA-4 cDNA was PCR amplified from human thymus cDNA library (Stratagene) and subcloned into pIRESneo (Clontech). A mutation of CTLA-4 that results in constitutive cell surface expression was introduced using MatchMaker Mutagenesis System (Promega). Mutation of tyrosine, Y201 to valine inhibits binding of the adaptin protein AP50 that is responsible for the rapid internalization of CTLA-4 (Chuang et al. *J. Immunol.* 159:144-151 (1997)). Mycoplasma-free 300.19 murine lymphoma cells were cultured in RPMI-1640 containing 10% fetal calf serum, non-essential amino acids, penicillin/streptomycin, 2mM glutamine, 12.5mM Hepes pH 7.5, and 25uM beta-mercaptoethanol. Cells were electroporated ( $3 \times 10^6$ /0.4ml serum free RPMI) in a 1ml chamber with 20ug CTLA-4-Y201V/pIRESneo using 200V/1180uF (Gibco CellPorator). Cells were rested for 10 minutes and then 8mls of prewarmed complete RPMI media. At 48 hours cells were diluted to  $0.5 \times 10^6$ /ml in complete RPMI media containing 1mg/ml G418 (Gibco). Resistant cells were expanded and shown to express CTLA-4 on the cell surface using the BNI3 antibody conjugated with phycoerythrin (PharMingen). High level expressing cells were isolated by sterile sorting.

Immunization and hybridoma generation: XenoMouse mice (8 to 10 weeks old) were immunized (i) subcutaneously at the base of tails with  $1 \times 10^7$  300.19 cells that were transfected to express CTLA-4 as described above, resuspended in phosphate buffered saline (PBS) with complete Freund's adjuvant, or (ii) subcutaneously at the base of tail with (a) 10  $\mu$ g the CTLA-4 fusion protein or (b) 10  $\mu$ g CTLA-4 peptide, emulsified with complete Freund's adjuvant. In each case, the dose was repeated three or four times in incomplete Freund's adjuvant. Four days before fusion, the mice received a final injection of the immunogen or cells in PBS. Spleen and/or lymph node lymphocytes from immunized mice were fused with the [murine non-secretory myeloma P3 cell line] and were subjected to HAT selection as previously described (Galfre, G. and Milstein, C., "Preparation of monoclonal antibodies: strategies and

procedures." *Methods Enzymol.* 73:3-46 (1981)). A large panel of hybridomas all secreting CTLA-4 specific human IgG<sub>2</sub>κ or IgG<sub>4</sub>κ (as detected below) antibodies were recovered.

**ELISA assay:** ELISA for determination of antigen-specific antibodies in mouse serum and in hybridoma supernatants was carried out as described (Coligan et al., Unit 2.1, "Enzyme-linked immunosorbent assays," in *Current protocols in immunology* (1994)) using CTLA-4-Ig fusion protein to capture the antibodies. For animals that are immunized with the CTLA-4-Ig fusion protein, we additionally screen for non-specific reactivity against the human Ig portion of the fusion protein. This is accomplished using ELISA plates coated with human IgG1 as a negative control for specificity.

In a preferred ELISA assay, the following techniques are used:

ELISA plates are coated with 100 µl/well of the antigen in plate coating buffer (0.1 M Carbonate Buffer, pH 9.6 and NaHCO<sub>3</sub> (MW 84) 8.4g/L). Plates are then incubated at 4°C overnight. After incubation, coating buffer is removed and the plate is blocked with 200 µl/well blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) and incubated at room temperature for 1 hour. Alternatively, the plates are stored in refrigerator with blocking buffer and plate sealers. Blocking buffer is removed and 50 µl/well of hybridoma supernatant, serum or other hybridoma supernatant (positive control) and HAT media or blocking buffer (negative control) is added. The plates are incubated at room temperature for 2 hours. After incubation, the plate is washed with washing buffer (1x PBS). The detecting antibody (i.e., mouse anti-human IgG2-HRP (SB, #9070-05) for IgG2 antibodies or mouse anti-human IgG4-HRP (SB #9200-05) for IgG4 antibodies) is added at 100µl/well (mouse anti-human IgG2-HRP @ 1:2000 or mouse anti-human IgG4-HRP @ 1:1000 (each diluted in blocking buffer)). The plates are incubated at room temperature for 1 hour and then washed with washing buffer. Thereafter, 100 µl/well of freshly prepared developing solution (10 ml Substrate buffer, 5 mg OPD (o-phenylenediamine, Sigma Cat No. P-7288), and 10 µl 30% H<sub>2</sub>O<sub>2</sub> (Sigma)) is added to the wells. The plates are allowed to develop 10-20 minutes, until

negative control wells barely start to show color. Thereafter, 100  $\mu$ l/well of stop solution (2 M  $\text{H}_2\text{SO}_4$ ) is added and the plates are read on an ELISA plate reader at wavelength 490 nm.

**Determination of affinity constants of fully human Mabs by BIAcore:**

Affinity measurement of purified human monoclonal antibodies, Fab fragments, or hybridoma supernatants by plasmon resonance was carried out using the BIAcore 2000 instrument, using general procedures outlined by the manufacturers.

Kinetic analysis of the antibodies was carried out using antigens immobilized onto the sensor surface at a low density. Three surfaces of the BIAcore sensorchip were immobilized with the CTLA-4-Ig fusion protein at a density ranging from approximately 390-900 using CTLA-4-Ig fusion protein at 20 or 50  $\mu$ g/ml in 10 mM sodium acetate at pH 5.0 using the amine coupling kit supplied by the manufacturer (BIAcore, Inc.). The fourth surface of the BIAcore sensorchip was immobilized with IgG1 (900 RU) and was used as a negative control surface for non-specific binding. Kinetic analysis was performed at a flow rate of 25 or 50 microliters per minute and dissociation ( $k_d$  or  $k_{off}$ ) and association ( $k_a$  or  $k_{on}$ ) rates were determined using the software provided by the manufacturer (BIA evaluation 3.0) that allows for global fitting calculations.

**EXAMPLE 2**

**Affinity Measurement of Anti-CTLA-4-Antibodies**

In the following Table, affinity measurements for certain of the antibodies selected in this manner are provided:

TABLE I

Hybridoma	Solid Phase (by BIAcore)				
	On-rates $K_a$ ( $M^{-1}S^{-1} \times 10^6$ )	Off-rates $K_d$ ( $S^{-1} \times 10^{-4}$ )	Association Constant $K_A$ (1/M)= $k_a/k_d \times 10^{10}$	Dissociation Constant $K_D$ (M)= $K_d/k_a \times 10^{-10}$	Surface Density [RU]
Moab01	0.68	1.01	0.67	1.48	878.7
	0.70	4.66	0.15	6.68	504.5
	0.77	6.49	0.19	8.41	457.2
	0.60	3.08	0.20	5.11	397.8
4.1.1	1.85	0.72	2.58	0.39	878.7
	1.88	1.21	1.55	0.64	504.5
	1.73	1.54	1.13	0.88	457.2
	1.86	1.47	1.26	0.79	397.8
4.8.1	0.32	0.07	4.46	0.22	878.7
	0.31	0.23	1.33	0.75	504.5
	0.28	0.06	4.82	0.21	397.8
4.14.3	2.81	3.04	0.92	1.08	878.7
	2.88	3.97	0.73	1.38	504.5
	2.84	6.66	0.43	2.35	457.2
	3.17	5.03	0.63	1.58	397.8
6.1.1	0.43	0.35	1.21	0.83	878.7
	0.46	0.90	0.51	1.98	504.5
	0.31	0.51	0.61	1.63	457.2
	0.45	0.79	0.57	1.76	397.8
3.1.1	1.04	0.96	1.07	0.93	878.7
	0.95	1.72	0.55	1.82	504.5
	0.73	1.65	0.44	2.27	457.2
	0.91	2.07	0.44	2.28	397.8
4.9.1	1.55	13.80	0.11	8.94	878.7
	1.43	19.00	0.08	13.20	504.5
	1.35	20.50	0.07	15.20	397.8
4.10.2	1.00	2.53	0.39	2.54	878.7
	0.94	4.30	0.22	4.55	504.5
	0.70	5.05	0.14	7.21	457.2
	1.00	5.24	0.19	5.25	397.8
2.1.3	1.24	9.59	0.13	7.72	878.7
	1.17	13.10	0.09	11.20	504.5
	1.11	13.00	0.09	11.70	397.8
4.13.1	1.22	5.83	0.21	4.78	878.7
	1.29	6.65	0.19	5.17	504.5
	1.23	7.25	0.17	5.88	397.8

As will be observed, antibodies prepared in accordance with the invention possess high affinities and binding constants.

### EXAMPLE 3

#### Structures of Anti-CTLA-4 Antibodies Prepared in Accordance with the Invention

In the following discussion, structural information related to antibodies prepared in accordance with the invention is provided.

In order to analyze structures of antibodies produced in accordance with the invention, we cloned genes encoding the heavy and light chain fragments out of the particular hybridoma. Gene cloning and sequencing was accomplished as follows:

Poly(A)<sup>+</sup> mRNA was isolated from approximately  $2 \times 10^5$  hybridoma cells derived from immunized XenoMouse mice using a Fast-Track kit (Invitrogen). The generation of random primed cDNA was followed by PCR. Human V<sub>H</sub> or human V<sub>κ</sub> family specific variable region primers (Marks et al., "Oligonucleotide primers for polymerase chain reaction amplification of human immunoglobulin variable genes and design of family-specific oligonucleotide probes." *Eur. J. Immunol.* 21:985-991 (1991)) or a universal human V<sub>H</sub> primer, MG-30 (CAGGTGCAGCTGGAGCAGTCIGG) was used in conjunction with primers specific for the human Cγ2 constant region (MG-40d; 5'-GCTGAGGGAGTAGAGTCCTGAGGA-3') or Cκ constant region (hκP2; as previously described in Green et al., 1994). Sequences of human Mabs-derived heavy and kappa chain transcripts from hybridomas were obtained by direct sequencing of PCR products generated from poly(A)<sup>+</sup> RNA using the primers described above. PCR products were also cloned into pCRII using a TA cloning kit (Invitrogen) and both strands were sequenced using Prism dye-terminator sequencing kits and an ABI 377 sequencing machine. All sequences were analyzed by alignments to the "V BASE sequence directory"

(Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK) using MacVector and Geneworks software programs.

Further, each of the antibodies 4.1.1, 4.8.1, 11.2.1, and 6.1.1 were subjected to full length DNA sequences. For such sequencing, Poly(A)<sup>+</sup> mRNA was isolated from approximately 4 X 10<sup>6</sup> hybridoma cells using mRNA Direct kit (Dynal). The mRNA was reverse transcribed using oligo-dT(18) and the Advantage RT/PCR kit (Clontech). The Variable region database (V Base) was used to design amplification primers beginning at the ATG start site of the heavy chain DP50 gene (5'-

TATCTAAGCTTCTAGACTCGACCGCCACCATGGAGTTTGGGCTGAGC TG-3') and to the stop codon of the IgG2 constant region (5'- TTCTCTGATCAGAATTCTATCATTTACCCGGAGACAGGGAGAGCT- 3'). An optimal Kozak sequence (ACCGCCACC) was added 5' to the ATG start site. The same method was used to design a primer to the ATG start site of the kappa chain A27 gene (5'- TCTTCAAGCTTGCCCGGGCCCGCCACCATGGAAACCCAGCGCAG -3') and the stop codon of the kappa constant region (5'- TTCTTTGATCAGAATTCTCACTAACACTCTCCCCTGTTGAAGC-3').

The 012 cDNA was cloned by using a primer to the ATG start site (5'- TCTTCAAGCTTGCCCGGGCCCGCCACCATGGACATGAGGGTCCCCGC T-3) and the kappa constant region stop codon primer above. The heavy chain cDNAs were also cloned as genomic constructs by site directed mutagenesis to add an NheI site at the end of the variable J domain and subcloning an NheI-fragment containing the genomic IgG2 CH1/Hinge/CH2/CH3 regions. The point mutation to generate NheI site does not alter the amino acid sequence from germline. The primer pairs were used to amplify the cDNAs using Advantage High Fidelity PCR Kit (Clontech). Sequence of the PCR was obtained by direct sequencing using dye-terminator sequencing kits and an ABI sequencing machine. The PCR product was cloned into pEE glutamine synthetase mammalian expression vectors (Lonza) and three clones were sequenced to confirm somatic mutations. For each clone, the sequence was verified on both strands in at least three reactions. An aglycosylated 4.1.1

antibody was generated by site directed mutagenesis of N294Q in the CH2 domain. Recombinant antibodies were produced by transient transfection of Cos7 cells in IgG depleted FCS and purified using standard Protein A sepharose techniques. Stable transfectants were generated by electroporation of murine NSO cells and selection in glutamine free media. Recombinant 4.1.1 with or without glycosylation exhibited identical specificity and affinity for CTLA4 in the *in vitro* ELISA and BIAcore assays.

Gene Utilization Analysis

The following Table sets forth the gene utilization evidenced by selected hybridoma clones of antibodies in accordance with the invention:



**TABLE II**  
**Heavy and Light Chain Gene Utilization**

Clone	Heavy Chain			Kappa Light Chain	
	VH	D	JH	VK	JK
4.1.1	DP-50	DIR4 or DIR3	JH4	A27	JK1
4.8.1	DP-50	7-27	JH4	A27	JK4
4.14.3	DP-50	7-27	JH4	A27	JK3
6.1.1	DP-50	DIR5 or DIR5rc	JH4	A27	JK3
3.1.1	DP-50	3-3	JH6	012	JK3
4.10.2	DP-50	7-27	JH4	A27	JK3
2.1.3	DP-65	1-26	JH6	A10/A26	JK4
4.13.1	DP-50	7-27	JH4	A27	JK3
11.2.1	DP-50	D1-26	JH6	012	JK3
11.6.1	DP-50	D2-2 or D4	JH6	012	JK3
11.7.1	DP-50	D3-22 or D21-9	JH4	012	JK3
12.3.1.1	DP-50	D3-3 or DXP4	JH6	A17	JK1
12.9.1.1	DP-50	D6-19	JH4	A3/A19	JK4
4.9.1	DP-47	5-24 and/or 6-19	JH4	L5	JK1

As will be observed, antibodies in accordance with the present invention were generated with a strong bias towards the utilization of the DP-50 heavy chain variable region. The DP-50 gene is also referred to as a V<sub>H</sub> 3-33 family gene. Only one antibody that was selected on the basis of CTLA-4 binding and preliminary *in vitro* functional assays showed a heavy chain gene utilization other than DP-50. That clone, 2.1.3, utilizes a DP-65 heavy chain variable region and is an IgG4 isotype. The DP-65 gene is also referred to as a V<sub>H</sub> 4-31 family gene. On the other hand, the clone, 4.9.1, which possesses a DP-47 heavy chain variable region binds to CTLA-4 but does not inhibit binding to B7-1 or B7-2. In XenoMouse mice, there are more than 30 distinct functional

heavy chain variable genes with which to generate antibodies. Bias, therefore, is indicative of a preferred binding motif of the antibody-antigen interaction with respect to the combined properties of binding to the antigen and functional activity.

#### Mutation Analysis

As will be appreciated, gene utilization analysis provides only a limited overview of antibody structure. As the B-cells in XenoMouse animals stochastically generate V-D-J heavy or V-J kappa light chain transcripts, there are a number of secondary processes that occur, including, without limitation, somatic hypermutation, n-additions, and CDR3 extensions. *See, for example,* Mendez et al. *Nature Genetics* 15:146-156 (1997) and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996. Accordingly, to further examine antibody structure predicted amino acid sequences of the antibodies were generated from the cDNAs obtained from the clones. In addition, N-terminal amino acid sequences were obtained through protein sequencing.

Figure 1 provides nucleotide and predicted amino acid sequences of the heavy and kappa light chains of the clones 4.1.1 (Figure 1A), 4.8.1 (Figure 1B), 4.14.3 (Figure 1C), 6.1.1 (Figure 1D), 3.1.1 (Figure 1E), 4.10.2 (Figure 1F), 2.1.3 (Figure 1G), 4.13.1 (Figure 1H), 11.2.1 (Figure 1I), 11.6.1 (Figure 1J), 11.7.1 (Figure 1K), 12.3.1.1 (Figure 1L), and 12.9.1.1 (Figure 1M). In Figures 1A, 1B, and 1D, extended sequences of the antibodies 4.1.1, 4.8.1, and 6.1.1 were obtained by full length cloning of the cDNAs as described above. In such Figures, the signal peptide sequence (or the bases encoding the same) are indicated in bold and sequences utilized for the 5' PCR reaction are underlined.

Figure 2 provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1, 4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence. Differences between the DP-50 germline sequence and that of the sequence in the clones are indicated in bold. The

Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibodies as shaded.

Figure 3 provides a sequence alignment between the predicted heavy chain amino acid sequence of the clone 2.1.3 and the germline DP-65 (4-31) amino acid sequence. Differences between the DP-65 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 4 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 4.10.2, and 4.13.1 and the germline A27 amino acid sequence. Differences between the A27 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined. Apparent deletions in the CDR1s of clones 4.8.1, 4.14.3, and 6.1.1 are indicated with "0s".

Figure 5 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 3.1.1, 11.2.1, 11.6.1, and 11.7.1 and the germline 012 amino acid sequence. Differences between the 012 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 6 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 2.1.3 and the germline A10/A26 amino acid sequence. Differences between the A10/A26 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 7 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.3.1 and the germline A17 amino acid sequence. Differences between the A17 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the

positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 8 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.9.1 and the germline A3/A19 amino acid sequence. Differences between the A3/A19 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 22 provides a series of additional nucleotide and amino acid sequences of the following anti-CTLA-4 antibody chains:

**4.1.1:**

full length 4.1.1 heavy chain (cDNA 22(a), genomic 22(b), and amino acid 22(c));

full length aglycosylated 4.1.1 heavy chain (cDNA 22(d) and amino acid 22(e));

4.1.1 light chain (cDNA 22(f) and amino acid 22(g));

**4.8.1:**

full length 4.8.1 heavy chain (cDNA 22(h) and amino acid 22(i));

4.8.1 light chain (cDNA 22(j) and amino acid 22(k));

**6.1.1:**

full length 6.1.1 heavy chain (cDNA 22(l) and amino acid 22(m));

6.1.1 light chain (cDNA 22(n) and amino acid 22(o));

**11.2.1:**

full length 11.2.1 heavy chain (cDNA 22(p) and amino acid 22(q)); and

11.2.1 light chain (cDNA 22 (r) and amino acid 22(s)).

5                   Signal peptide sequences are shown in bold and large text. The open  
reading frames in the full length 4.1.1 genomic DNA sequence (Fig. 22(b)) are  
underlined. And, the mutations introduced to make the aglycosylated 4.1.1  
10 heavy chain and the resulting change (N294Q) are shown in double underline and  
5 bold text (cDNA (Fig. 22(b)) and amino acid (Fig. 22(c))).

#### EXAMPLE 4

##### Analysis of Heavy and Light Chain Amino Acid Substitutions

10           In Figure 2, which provides a sequence alignment between the predicted  
heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1,  
20 3.1.1, 4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the  
germline DP-50 (3-33) amino acid sequence, an interesting pattern emerges. In  
addition to the fact of the bias for heavy chain DP-50 in the majority of the  
25 clones, there is relatively limited hypermutation in the antibodies relative to the  
germline DP-50 gene. For example, clones 3.1.1 and 11.2.1 have no mutations.  
Moreover, the mutations in the other clones are generally conservative changes,  
involving substitutions of amino acids with similar properties to the amino acids  
30 in the germline. Mutations within many of the CDR1 and CRD2 sequences are  
particularly conservative in nature. Three of the heavy chains represented in  
Figure 2, 4.10.2, 4.13.1, and 4.14.3, are clearly derived from a single  
35 recombination event (i.e., derive from an identical germinal center) and are  
nearly identical in sequence. If these three are considered as a single sequence,  
then, among the 10 different antibodies containing the DP50 heavy chain, in  
40 25 CDR1 and CDR2 there are 3 positions in which a nonpolar residue is replaced  
by another nonpolar residue, 12 in which a polar uncharged residue is replaced  
by another polar uncharged residue, and 1 in which a polar charged residue is  
replaced by another polar charged residue. Further, there are two positions in  
45 which two residues which are very similar structurally, glycine and alanine, are  
30 substituted for one another. The only mutations not strictly conservative  
involve 3 substitutions of a polar charged residue for a polar uncharged residue  
and one substitution of a nonpolar residue for a polar residue.

5 The light chains of these antibodies are derived from 5 different Vk genes. The A27 gene is the most heavily represented and is the source of 6 different light chains. Comparison of these 6 sequences reveals two noteworthy features. First, in three of them, 4.8.1, 4.14.3, and 6.1.1, contain deletions of 10 one or two residues in CDR1, a rare event. Second, there is a strong prejudice against the germline serine at position six in CDR3 in that the serine has been replaced in every sequence. This suggests that a serine at this position is 15 incompatible with CTLA4 binding.

It will be appreciated that many of the above-identified amino acid 10 substitutions exist in close proximity to or within a CDR. Such substitutions would appear to bear some effect upon the binding of the antibody to the 20 CTLA-4 molecule. Further, such substitutions could have significant effect upon the affinity of the antibodies.

#### 25 **EXAMPLE 5**

#### **N-Terminal Amino Acid Sequence Analysis of Antibodies in Accordance with the Invention**

30 In order to further verify the composition and structure of the antibodies 20 in accordance with the invention identified above, we sequenced certain of the antibodies using a Perkin-Elmer sequencer. Both heavy and kappa light chains 35 of the antibodies were isolated and purified through use of preparative gel electrophoresis and electroblotting techniques and thereafter directly sequenced as described in Example 6. A majority of the heavy the heavy chain sequences 40 were blocked on their amino terminus. Therefore, such antibodies were first treated with pyroglutamate aminopeptidase and thereafter sequenced.

45 The results from this experiment are shown in Figure 9. Figure 9 also provides the molecular weight of the heavy and light chains as determined by mass spectroscopy (MALDI).

**EXAMPLE 6****Additional Characterization of Antibodies in Accordance with the  
Invention**

Figure 10 provides certain additional characterizing information about certain of the antibodies in accordance with the invention. In the Figure, data related to clones 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.14.3, and 6.1.1 is summarized. The following data is provided: Concentration, isoelectric focusing (IEF), SDS-PAGE, size exclusion chromatography, FACS, mass spectroscopy (MALDI), and light chain N-terminal sequences.

Generally, the data was generated as follows:

**Materials and Methods**

Protein concentration was determined at 280 nm from a UV scan (200-350 nm), where 1.58 absorbance units at 280 nm equaled 1mg/ml.

SDS-PAGE was performed using the Novex NuPAGE electrophoresis system with a 10% NuPAGE gel and MES running buffer. Samples were prepared by diluting 3:1 with 4x NuPAGE sample buffer (+/-) beta-mercaptoethanol, heated and ~ 5 ug of protein was loaded onto the gel. The gel was then stained with Brilliant Blue R staining solution (Sigma cat.# B-6529) and molecular size estimates were made by comparing stained bands to "Perfect Protein Markers" (Novagen cat# 69149-3).

For N-terminal sequencing, samples were run as above on NuPAGE gels, transferred to Pro Blot immobilization membrane (Applied Biosystems) then stained with Coomassie Blue R-250. The stained protein bands were excised and subjected to sequence analysis by automated Edman degradation on an Applied Biosystems 494 Procisc HT Sequencer.

5                    Isoelectric focusing (IEF) was performed using Pharmacia IEF 3-9  
pHast gels (cat# 17-0543-01) . Samples were diluted in 10% glycerol to ~0.8  
10                    mg/ml and 1ul was loaded onto gel and then silver stained. The pI estimates  
were made by comparing stained bands to broad range (pH3-10) IEF standards  
5                    (Pharmacia cat # 17-0471-01)

15                    Size exclusion chromatography (SEC) was carried in phosphate buffered  
saline (PBS) on the Pharmacia SMART system using the Superdex 75 PC  
3.2/30 column. Molecular size estimates were made by comparing peak  
10                    retention time to the retention times of gel

20                    For FACS studies, human peripheral T cells were prepared and  
stimulated for 48 hours. T cells were washed once, resuspended in FACS  
buffer at  $1 \times 10^6$  cells/100 ul and stained for CD3 surface expression with 10 ul  
25                    15 of anti-CD3-FITC (Immunotech, Marseille, France) for 30 minutes at room  
temperature. Cells were washed twice, then fixed, permeabilized (Fix and Perm,  
Caltag), and stained for intracellular CTLA-4 expression with 10 ul anti-  
30                    CD152-PE (Pharmingen). Flow cytometry was performed using a Becton  
Dickinson FACSort. Quadrants were set by analysis of relevant isotype control  
20                    antibodies (Caltag).

35                    As was discussed above, anti-CTLA-4 antibodies have been  
demonstrated to possess certain powerful immune modulation activities. The  
40                    25 following experiments were carried out in order to determine if antibodies in  
accordance with the present invention possessed such activities. In general, the  
experiments were designed to assess ability of the antibodies to inhibit the  
interaction between CTLA-4 and B7 molecules, be selective as between CTLA-  
45                    4 and B7 molecules and CD28, and promote T cell cytokine production,  
30                    including, but not limited to IL-2 and/or IFN- $\gamma$  expression. Further,  
examination of cross-reactivity of antibodies of the invention with certain



human tissues and CTLA-4 molecules in other species (e.g., mouse and primate) was undertaken.

#### EXAMPLE 7

##### **Competition ELISA: Inhibition of CTLA-4/B7-1 or B7-2 Interaction by Antibodies in Accordance with the Invention**

An *in vitro* assay was conducted to determine if antibodies in accordance with the present invention were capable of inhibiting the binding of CTLA-4 with either B7-1 or B7-2. As will be appreciated, antibodies of the invention that are capable of inhibiting the binding of CTLA-4 with B7 molecules would be expected to be candidates for immune regulation through the CTLA-4 pathway. In the assay, the following materials and methods were utilized:

#### **Materials and Methods**

3 nM B7.1-Ig(G1) or B7.2-Ig(G1) (Repligen, Inc. Needham, MA) in Dulbecco's PBS was coated on 96-well MaxiSorp plates (Nunc, Denmark, #439454) and incubated at 4°C overnight. On day 2, B7-Ig was removed and plates were blocked with 1% BSA plus 0.05% Tween-20 in D-PBS for two hours. Plates were washed 3X with wash buffer (0.05% Tween-20 in D-PBS). Antibody at appropriate test concentrations and CTLA-4-Ig(G4) (0.3 nM final conc.) (Repligen, Inc. Needham, MA) were pre-mixed for 15 minutes and then added to the B7-Ig coated plate (60 µl total volume) and incubated at RT for 1.5 hours. Plates were washed 3X and 50 µl of a 1 to 1000 dilution of HRP-labeled mouse anti-human IgG4 antibody (Zymed, San Francisco, CA, #05-3820) was added and incubated at RT for 1 hour. Plates were washed 3X and 50 µl TMB Microwell peroxidase substrate (Kirkegaard & Perry, Gaithersburg, MD, #50-76-04) was added and incubated at RT for 20 minutes, and then 50 µl 1N H<sub>2</sub>SO<sub>4</sub> was added to the plate. Plates were read at 450 nm using a Molecular Devices plate reader (Sunnyvale, CA). All samples were tested in duplicate. Maximal

signal was defined as CTLA-4-Ig binding in the absence of test antibody. Non-specific binding was defined as absorbance in the absence of CTLA-4-Ig and test antibody.

The results from the assay are provided in Table IIIA and IIIB. In Table IIIA, results are shown for a variety of antibodies in accordance with the invention. In Table IIIB, results are shown comparing the 4.1.1 antibody of the invention with the 11.2.1 antibody of the invention from a separate experiment.

**TABLE IIIA**

Clone CTLA-4-Ig	Isotype	CTLA4/B7.2 Comp. ELISA IC50 (nM)	CTLA4/B7.1 Comp. ELISA IC50 (nM)
CT3.1.1	IgG2	0.45 ± 0.07 (n=3)	0.63 ± 0.10 (n=2)
CT4.1.1	IgG2	0.38 ± 0.06 (n=5)	0.50 ± 0.05 (n=2)
CT4.8.1	IgG2	0.57 ± 0.03 (n=3)	0.17 ± 0.28 (n=2)
CT4.9.1	IgG2	Non-competitive (n=3)	non-competitive (n=2)
CT4.10.2	IgG2	1.50 ± 0.37 (n=3)	3.39 ± 0.31 (n=2)
CT4.13.1	IgG2	0.49 ± 0.05 (n=3)	0.98 ± 0.11 (n=2)
CT4.14.3	IgG2	0.69 ± 0.11 (n=3)	1.04 ± 0.15 (n=2)
CT6.1.1	IgG2	0.39 ± 0.06 (n=3)	0.67 ± 0.07 (n=2)

**TABLE IIIB**

Clone CTLA-4-Ig	Isotype	CTLA4/B7.2 Comp. ELISA IC50 (nM)	CTLA4/B7.1 Comp. ELISA IC50 (nM)
CT4.1.1	IgG2	0.55 ± 0.08 (n=4)	0.87 ± 0.14 (n=2)
CT11.2.1	IgG2	0.56 ± 0.05 (n=4)	0.81 ± 0.24 (n=2)

**EXAMPLE 8****Selectivity Ratios of Antibodies of the Invention with Respect to CTLA-4  
Versus Either CD28 or B7-2**

Another *in vitro* assay was conducted to determine the selectivity of antibodies of the invention with respect to CTLA-4 versus either CD28 or B7-2. The following materials and methods were utilized in connection with the experiments:

**CTLA-4 Selectivity ELISA: Materials and Methods**

A 96-well FluroNUNC plate (Nunc Cat No.475515) was platecoated with four antigens: CTLA-4/Ig, CD44/Ig, CD28/Ig, and B7.2/Ig (antigens generated in-house). The antigens were platecoated overnight at +4°C at 1ug/ml 100ul/well in 0.1M sodium bicarbonate buffer, pH 9.6. The plate was then washed with PBST (PBS + 0.1% Tween-20) three times using a NUNC plate washer. The plate was blocked with PBST+0.5%BSA at 150 ul/well. The plate was incubated at RT for 1 hour then washed with PBST three times. Next the anti-CTLA-4 antibodies of the invention were diluted in block at 1 µg/ml and were added to the plate. The plate was incubated at RT for 1 hour then washed with PBST three times. The wells that contained the antibodies of the invention were then treated with 100 µl/well anti-human IgG2-HRP (Southern Biotech Cat No.9070-05) at a 1:4000 dilution in block. Also, one row was treated with anti-human IgG (Jackson Cat No. 209-035-088) to normalize for platecoating. This antibody was diluted to 1:5000 in block and added at 100 ul/well. Also, one row was treated with anti-human CTLA-4-HRP (Pharmingen Cat No. 345815/Custom HRP conjugated) as a positive control. This antibody was used at 0.05 ug/ml diluted in block. The plate was incubated at RT for 1 hour then washed with PBST three times. LBA chemiluminescent substrate (Pierce) was added at 100 µl/well and the plate was incubated on a plateshaker for 5 min. The plate was then read using a lumi-imager for a 2 min. exposure.

**IGEN CTLA-4-Ig Selectivity Binding Assay: Materials and Methods**

M-450 Dynabeads (Dynal A.S, Oslo, Norway #140.02) were washed 3X with Na phosphate buffer, pH 7.4 and resuspended in Na phosphate buffer. 1.0 µg CTLA-4-Ig(G1), 1.0 µg CD28-Ig(G1) or 1.0 to 3.0 µg B7.2-Ig(G1) (Repligen, Inc. Needham, MA) were added to 100 µl of beads and incubated overnight on a rotator at 4°C. On day 2 the beads were washed 3X in 1% BSA plus 0.05% Tween-20 in Dulbecco's PBS and blocked for 30 minutes. Beads were diluted 1 to 10 with blocking buffer and 25 µl of the coated beads were added to 12x75 mm polypropylene tubes. All samples were tested in duplicate. 50 µl test antibody (1 µg/ml final concentration) or blocking buffer was added to the tubes and incubated for 30 minutes on the Origen 1.5 Analyzer carousel (IGEN International, Inc., Gaithersburg, MD) at RT, vortexing at 100 rpm. 25 µl of ruthenylated murine anti-human IgG1, IgG2 or IgG4 (Zymed, Inc. San Francisco, CA #05-3300, 05-3500 and 05-3800) (final concentration of 3 µg/ml in 100 µl total volume) was added to the tubes. Tubes were incubated for 30 minutes at RT on the carousel vortexing at 100 rpm. 200 µl of Origen assay buffer (IGEN International, Inc., Gaithersburg, MD #402-050-03) per tube was added and briefly vortexed and then the tubes were counted in the Origen Analyzer and ECL (electrochemiluminescence) units were determined for each tube. Normalization factors were determined to correct for differences in binding of fusion proteins to Dynabeads, and ECL units were corrected for non-specific binding before calculating selectivity ratios.

The results from the assays are provided in Tables IVA and IVB.

**TABLE IVA**

Clone	Isotype	CTLA4/CD28 ELISA	CTLA4/B7.2 ELISA	CTLA4/CD44 ELISA	CTLA4/CD28 IGEN	CTLA4/B7.2 IGEN
3.1.1	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=1) 195:1 (n=1)
4.1.1	IgG2	>500:1 (n=3)	>500:1 (n=2) 485:1 (n=1)	>500:1 (n=3)	>500:1 (n=1) 261:1 (n=1)	>500:1 (n=1) 107:1 (n=1)
4.8.1	IgG2	>500:1 (n=3)	>500:1 (n=2) 190:1 (n=1)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=2)
4.9.1	IgG2	>500:1 (n=2) 244:1 (n=1)	>500:1 (n=2) 33:1 (n=1)	>500:1 (n=3)	>500:1 (n=1)	>500:1 (n=1)
4.10.2	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=1)	>500:1 (n=1)
4.13.1	IgG2	>500:1 (n=2) 46:1 (n=1)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=1) 329:1 (n=1)	>500:1 (n=2)
4.14.3	IgG2	>500:1 (n=2) 80:1 (n=1)	>500:1 (n=2) 10:1 (n=1)	>500:1 (n=2) 126:1 (n=1)	>413:1 (n=1)	>234:1 (n=1)
6.1.1	IgG2	>500:1 (n=2) 52:1 (n=1)	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=2)

**TABLE IVB**

Clone	Isotype	CTLA4/CD28 ELISA	CTLA4/B7.2 ELISA	CTLA4/hIgG ELISA
4.1.1	IgG2	>500:1 (n=3)	>500:1 (n=2)	>500:1 (n=3)
11.2.1	IgG2	>500:1 (n=3)	>500:1 (n=3)	>500:1 (n=3)

**EXAMPLE 9****Human T-Cell Signal Model**

In order to further define the activity of antibodies in accordance with the invention to act as immune regulators, we developed certain T-cell assays in order to quantify the enhancement of T-cell IL-2 production upon blockade of

CTLA-4 signal with the antibodies. The following materials and methods were utilized in connection with the experiments:

#### Materials and Methods

Freshly isolated human T cells were prepared by using Histopaque (Sigma, St. Louis, MO #A-70543) and T-kwik (Lympho-Kwik, One Lambda, Canoga Park, CA, #LK-50-T), and stimulated with PHA (1  $\mu$ g/ml) (Purified Phytohemagglutinin, Murex Diagnostics Ltd. Dartford, England, #HA 16) in medium (RPMI 1640 containing L-glutamine, MEM non-essential amino acids, penicillin, streptomycin, 25 mM Hepes and 10% FBS) at a concentration of  $1 \times 10^6$  cells/ml and incubated at 37°C for 2 days. The cells were washed and diluted in medium to  $2 \times 10^6$  cells/ml. Raji cells (Burkitt lymphoma, Human ATCC No.: CCL 86 Class II American Type Culture Collection Rockville, MD) were treated with mitomycin C (Sigma St. Louis, MO, # M-4287) (25  $\mu$ g/ml) for one hour at 37°C. The Raji cells were washed 4X in PBS and resuspended at  $2 \times 10^6$  cells/ml. Human T cell blasts ( $5 \times 10^5$ /ml), Raji cells ( $5 \times 10^5$ /ml) and anti-CTLA-4 antibodies or an isotyped-matched control antibody at various concentrations were added to 96-well microtiter plates and the plates were incubated at 37°C for 72 hours. Total volume per well was 200  $\mu$ l. Seventy-two hours post stimulation, the plates were spun down and supernatant removed and frozen for later determination of IL-2 (Quantikine IL-2 ELISA kit, R&D Systems, Minneapolis, MN, #D2050) and IFN- $\gamma$  (Quantikine IFN-g ELISA kit, R&D Systems). Cytokine enhancement was defined as the difference between cytokine levels in cultures containing an anti-CTLA-4 blocking mAb versus an isotype-matched control antibody. For flow cytometry experiments, Raji cells were washed 1x with FACS buffer (PBS containing 2% heat inactivated FCS, 0.025% sodium azide). Cell pellets were resuspended in FACS buffer at  $1 \times 10^6$  cells/100  $\mu$ l and incubated with 10  $\mu$ l of anti-CD80-PE (Becton Dickinson, San Jose, CA) or anti-CD86-PE (Pharmingen, San Diego, CA) for 30 minutes at room temperature. Cells were washed twice and resuspended in 1 ml FACS buffer. Flow cytometry was performed using a Becton Dickinson FACSsort.

5 Histogram markers were set by analysis of relevant isotype control antibodies (Caltag, Burlingame, CA).

10 In general, we have developed an assay that can be used for rapid  
5 determination of T-cell IL-2 upregulation. As will be appreciated, stimulation of T cells is B7 and CD28 dependent. Further, washed T blasts do not make detectable IL-2 and Raji cells do not make detectable IL-2 even when  
15 stimulated with LPS or PWM. However, in combination, the T blasts co-cultured with Raji cells can model B7, CTLA-4, and CD28 signaling events and  
10 the effects of antibodies thereon can be assessed.

20 Figure 11 shows the expression of B7-1 and B7-2 on Raji cells using anti-CD80-PE and anti-CD86-PE mAbs using flow cytometry (FACs) as described in Example 6.

25 Figure 12 shows the concentration dependent enhancement of IL-2  
15 production in the T cell blast/Raji assay induced by CTLA-4 blocking antibodies (BNI3 (PharMingen) and the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention).

30 Figure 13 shows the concentration dependent enhancement of IFN- $\gamma$   
production in the T cell blast/Raji assay induced by CTLA-4 blocking  
20 antibodies (BNI3 (PharMingen) and the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention) (same donor T cells).

35 Figure 14 shows the mean enhancement of IL-2 production in T cells from 6 donors induced by CTLA-4 blocking antibodies in the T cell blast/Raji assay. It is interesting to consider that the mAb, CT4.9.1, binds to CTLA4 but  
25 does not block B7 binding. Thus, simply binding to CTLA-4 is insufficient by  
40 itself to provide a functional antibody of the invention.

45 Figure 15 shows the mean enhancement of IFN- $\gamma$  production in T cells from 6 donors induced by CTLA-4 blocking antibodies in the T cell blast/Raji assay.

30 Figure 19 shows a comparison between the 4.1.1 and 11.2.1 antibodies of the invention at a concentration of 30 $\mu$ g/ml in the 72 hour T cell blast/Raji

assay as described in this Example 9 and the Superantigen assay described in Example 10.

Figure 20 shows the concentration dependent enhancement of IL-2 production in the T cell blast/Raji assay induced by the 4.1.1 and 11.2.1 CTLA4 antibodies of the invention.

The following Table IVc provides information related to mean enhancement and range of enhancement of cytokine response in the Raji and SEA assays of the invention. Each of the experiments included in the results are based on antibody at a dose of 30 µg/ml and measured at 72 hours.

Numbers of donors used in the experiments as well as responses are shown.

**TABLE IVc**

Assay	mAb	Cytokine	Mean Enhancement pg/ml	SEM	Range Enhancement pg/ml	n	Donor Response
T cell blast/Raji	4.1.1	IL-2	3329	408	0 to 8861	42	19 of 21
T cell blast/Raji	4.1.1	IFN-γ	3630	980	600 to 13939	17	13 of 13
T cell blast/Raji	11.2.1	IL-2	3509	488	369 to 6424	18	14 of 14
SEA (PBMC)	4.1.1	IL-2	2800	312	330 to 6699	42	17 of 17
SEA (PBMC)	11.2.1	IL-2	2438	366	147 to 8360	25	15 of 15
SEA (Whole Blood)	4.1.1	IL-2	6089	665	-168 to 18417	46	15 of 17
SEA (Whole Blood)	11.2.1	IL-2	6935	700	-111 to 11803	25	12 of 14

#### **EXAMPLE 10**

##### **Human T-Cell Signal Model**

We developed a second cellular assay in order to quantify the enhancement of T-cell IL-2 upregulation upon blockade of CTLA-4 signal with the antibodies. The following materials and methods were utilized in connection with the experiments:



## Materials and Methods

Human PBMC were prepared using Accuspin. Microtiter plates were precoated with an anti-CD3 antibody (leu4, Becton Dickinson) (60 ng/ml) and incubated for 2 hours at 37°C. hPBMC were added to the wells at 200,000 cells per well. Staphylococcus enterotoxin A (SEA) (Sigma) was added to the wells at 100 ng/ml. Antibodies were added to the wells, usually at 30 µg/ml. Cells were then stimulated for 48, 72 or 96 hours. Plates were centrifuged at the desired time-point and supernatants were removed from the wells. Thereafter, supernatants were checked for IL-2 production using ELISA (R&D Systems).

Results from these experiments are shown in Figures 16, 17, and 21. In Figure 16, induction of IL-2 production in hPBMC from 5 donors was measured 72 hours after stimulation. In Figure 17, results are shown from measurement of whole blood, analyzing the difference in induction of IL-2 production in the blood of 3 donors as measured at 72 and 96 hours after stimulation.

In Figure 21, the enhancement of IL-2 production in whole blood of 2 donors as measured at 72 hours after stimulation.

### EXAMPLE 11

#### Tumor Animal Model

We have established an animal tumor model for the *in vivo* analysis of anti-murine-CTLA-4 antibodies in inhibiting tumor growth. In the model, a murine fibrosarcoma tumor is grown and the animals are treated with anti-murine-CTLA-4 antibodies. The materials and methods for establishment of the model are provided below:

## Materials and Methods

Female A/J mice (6-8 weeks old) were injected subcutaneously on the dorsal side of the neck with 0.2 ml of Sa1N tumor cells ( $1 \times 10^6$ ) (Baskar 1995).

5 Anti-murine CTLA-4 or an isotype matched control antibody (PharMingen, San Diego, CA, 200 ug/animal) were injected intraperitoneally on days 0, 4, 7 and 14 following the injection of tumor cells. Tumor measurements were taken during the course of the 3-4 week experiments using a Starrett SPC Plus  
10 electronic caliper (Athol, MA) and tumor size was expressed as the surface area covered by tumor growth (mm<sup>2</sup>).

15 Figure 18 shows the inhibition of tumor growth with an anti-murine CTLA-4 antibody in a murine fibrosarcoma tumor model. As shown in Figure 18, animals treated with anti-CTLA-4 had a reduction in tumor growth as compared to animals treated with an isotype control antibody. Accordingly,  
20 anti-murine CTLA4 mAbs are capable of inhibiting growth of a fibrosarcoma in a mouse tumor model.

It is expected that antibodies that are cross-reactive with murine CTLA-4 would perform similarly in the model. However, of the antibodies of the  
25 invention that have been checked for cross-reactivity, none are cross-reactive with murine CTLA-4.

#### 30 EXAMPLE 12

##### Tumor Animal Model

20 In order to further investigate the activity of antibodies in accordance with the invention, a xenograft SCID mouse model was designed to test the eradication of established tumors and their derived metastases. In the model, SCID mice are provided with grafted human T cells and are implanted with  
35 patient-derived non-small cell lung cell (NSCL) or colorectal carcinoma (CC) cells. Implantation is made into the gonadal fat pads of SCID mice. The tumors are allowed to grow, and thereafter removed. The mice develop human-like tumor and liver metastases. Such a model is described in Bumpers et al *J. Surgical Res.* 61:282-288 (1996).  
45

30 It is expected that antibodies of the invention will inhibit growth of tumors formed in such mice.

5

INCORPORATION BY REFERENCE

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5 All references cited herein, including patents, patent applications,  
papers, text books, and the like, and the references cited therein, to the extent  
that they are not already, are hereby incorporated herein by reference in their  
entirety. In addition, the following references are also incorporated by  
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**EQUIVALENTS**

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5       The foregoing description and Examples detail certain preferred  
embodiments of the invention and describes the best mode contemplated by the  
inventors. It will be appreciated, however, that no matter how detailed the  
15       foregoing may appear in text, the invention may be practiced in many ways and  
the invention should be construed in accordance with the appended claims and  
10       any equivalents thereof.

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CLAIMSWHAT WE CLAIM IS:

1. An antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V<sub>H</sub>3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or framework sequences shown in Figure 2.

2. The antibody of Claim 1, wherein the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70.

3. The antibody of Claim 1, further comprising a light chain variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

4. The antibody of Claim 2, further comprising a light chain variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ

5 ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,  
and SEQ ID NO:71.

10 5. An antibody comprising a heavy chain amino acid sequence  
5 comprising SEQ ID NO:1 and a light chain variable amino acid sequence  
comprising SEQ ID NO:14.

15 6. An antibody comprising a heavy chain amino acid sequence  
comprising SEQ ID NO:2 and a light chain variable amino acid sequence  
10 comprising SEQ ID NO:15.

20 7. An antibody comprising a heavy chain amino acid sequence  
comprising SEQ ID NO:4 and a light chain variable amino acid sequence  
comprising SEQ ID NO:17.

25 8. A isolated human monoclonal antibody that is capable of binding  
to CTLA-4.

30 9. The antibody of Claim 8, wherein the antibody is capable of  
20 competing for binding with CTLA-4 with an antibody selected from the group  
consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1,  
35 11.7.1, 12.3.1.1, and 12.9.1.1.

40 10. The antibody of Claim 8, wherein the antibody possesses a  
25 substantially similar binding specificity to CTLA-4 as an antibody selected from  
the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,  
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

45 11. The antibody of Claim 8, wherein the antibody is selected from  
30 the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1,  
11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1.

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5 12. The antibody of Claim 8, wherein the antibody is not cross reactive with CTLA-4 from lower mammalian species.

10 13. The antibody of Claim 12, wherein the lower mammalian species  
5 comprises mouse, rat, and rabbit.

15 14. The antibody of Claim 8, wherein the antibody is cross reactive with CTLA-4 from primates.

10 15. The antibody of Claim 14, wherein the primates comprise  
20 cynomolgous and rhesus monkeys.

25 16. The antibody of Claim 8, wherein the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than  
15 about 100:1.

30 17. The antibody of Claim 16, wherein the selectivity is about 500:1 or greater.

20 18. The antibody of Claim 8, wherein a binding affinity of the antibody is about  $10^{-9}$  M or greater.

35 19. The antibody of Claim 18, wherein a binding affinity of the antibody is about  $10^{-10}$  M or greater.

40 20. The antibody of Claim 8, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 100 nM.

45 21. The antibody of Claim 20, wherein the antibody inhibits binding  
30 between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 0.38 nM.

5                   22.    The antibody of Claim 8, wherein the antibody inhibits binding  
between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 100 nM or greater.

10                   23.    The antibody of Claim 22, wherein the antibody inhibits binding  
5   between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 0.50 nM.

15                   24.    The antibody of Claim 8, wherein the antibody enhances IL-2  
production in a T cell blast/Raji assay by about 500 pg/ml or greater.

10                   25.    The antibody of Claim 24, wherein the antibody enhances IL-2  
20   production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

25                   26.    The antibody of Claim 8, wherein the antibody enhances IFN- $\gamma$   
15   production in a T cell blast/Raji assay by about 500 pg/ml or greater.

30                   27.    The antibody of Claim 26, wherein the antibody enhances IFN- $\gamma$   
production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

35                   28.    The antibody of Claim 8, wherein the antibody induces IL-2  
20   production in a hPBMC or whole blood superantigen assay by about 500 pg/ml  
or greater.

40                   29.    The antibody of Claim 26, wherein the antibody induces IL-2  
25   production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml  
or greater

45                   30.    The antibody of Claim 26, wherein the antibody induces IL-2  
production in a hPBMC or whole blood superantigen assay by greater than  
about 30% relative to control.



5                   31. The antibody of Claim 26, wherein the antibody induces IL-2  
production in a hPBMC or whole blood superantigen assay by greater than  
about 50% relative to control.

10                   32. A humanized antibody that possesses a substantially similar  
5 binding specificity to CTLA-4 as an antibody selected from the group  
consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1,  
15 11.7.1, 12.3.1.1, and 12.9.1.1.

10                   33. The antibody of Claim 32, wherein the antibody is not cross  
20 reactive with CTLA-4 from lower mammalian species.

                  34. The antibody of Claim 33, wherein the lower mammalian species  
25 comprises mouse, rat, and rabbit.

15                   35. The antibody of Claim 32, wherein the antibody is cross reactive  
with CTLA-4 from primates.

30                   36. The antibody of Claim 35, wherein the primates comprise  
20 cynomolgous and rhesus monkeys.

35                   37. The antibody of Claim 32, wherein the antibody possesses a  
selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than  
about 100:1.

40                   38. The antibody of Claim 37, wherein the selectivity is about 500:1  
or greater.

45                   39. The antibody of Claim 32, wherein a binding affinity of the  
30 antibody is about  $10^{-9}$  M or greater.

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5                   40.    The antibody of Claim 39, wherein a binding affinity of the antibody is about  $10^{-10}$  M or greater.

10                   41.    The antibody of Claim 32, wherein the antibody inhibits binding  
5    between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 100 nM.

15                   42.    The antibody of Claim 41, wherein the antibody inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of lower than about 0.38 nM.

10                   43.    The antibody of Claim 32, wherein the antibody inhibits binding  
20    between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 100 nM or greater.

25                   44.    The antibody of Claim 43, wherein the antibody inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of lower than about 0.50 nM.

15                   45.    The antibody of Claim 32, wherein the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

30                   46.    The antibody of Claim 45, wherein the antibody enhances IL-2  
20    production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

35                   47.    The antibody of Claim 32, wherein the antibody enhances IFN- $\gamma$  production in a T cell blast/Raji assay by about 500 pg/ml or greater.

40                   48.    The antibody of Claim 47, wherein the antibody enhances IFN- $\gamma$   
25    production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

45                   49.    The antibody of Claim 32, wherein the antibody enhances IL-2  
30    production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater.

5 50. The antibody of Claim 49, wherein the antibody enhances IL-2  
production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml  
or greater

10 51. The antibody of Claim 32, wherein the antibody enhances IL-2  
production in a hPBMC or whole blood superantigen assay by greater than  
about 30% relative to control.

15 52. The antibody of Claim 50, wherein the antibody enhances IL-2  
10 production in a hPBMC or whole blood superantigen assay by greater than  
about 50% relative to control.

20 53. An antibody that binds to CTLA-4, comprising a heavy chain  
amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded  
25 by a human  $V_H$  3-33 gene family operably linked in frame with a CDR1, a  
CDR2, and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being  
independently selected from the CDR1, CDR2, and CDR3 sequences illustrated  
30 in Figure 2.

20 54. The antibody of Claim 32, further comprising any of the somatic  
mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2.

35 55. An antibody that binds to CTLA-4, comprising a heavy chain  
amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded  
40 by a human  $V_H$  3-33 gene family operably linked in frame with a CDR1, a  
CDR2, and a CDR3 sequence, which antibody has the following properties:

a binding affinity for CTLA-4 of about  $10^{-9}$  or greater;

inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of about

45 100 nM or lower;

30 inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of about

100 nM or lower; and

5 enhances cytokine production in an assay of human T cells by  
500 pg/ml or greater.

10 56. An antibody that binds to CTLA-4, comprising a heavy chain  
5 amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked  
in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected  
from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3,  
15 which antibody has the following properties:

a binding affinity for CTLA-4 of about  $10^{-9}$  or greater;

10 inhibits binding between CTLA-4 and B7-1 with an  $IC_{50}$  of about  
100 nM or lower;

20 inhibits binding between CTLA-4 and B7-2 with an  $IC_{50}$  of about  
100 nM or lower; and

25 enhances cytokine production in an assay of human T cells by  
15 500 pg/ml or greater.

30 57. A cell culture system for assaying T cell stimulation, comprising  
a culture of human T cell blasts co-cultured with a Raji cell line.

20 58. The cell culture system of Claim 57, wherein the T cell blasts are  
washed prior to culture with the Raji cell line.

35 59. An assay for measuring T cell stimulation, comprising:  
providing a culture of human T cell blasts and a Raji cell line;  
25 contacting the culture with an agent; and  
40 measuring cytokine production by the culture.

45 60. The assay of Claim 59, wherein the T cell blasts are washed prior  
to culture with the Raji cell line.

30 61. The assay of Claim 59, wherein the cytokine is IL-2.

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62. The assay of Claim 59, wherein the cytokine is IFN- $\gamma$ .

10

63. The assay of Claim 59, wherein cytokine production is measured in supernatant isolated from the culture.

5

64. The assay of Claim 59, wherein the agent is an antibody.

15

65. The assay of Claim 59, wherein the antibody binds to CTLA-4.

10

66. A functional assay for screening a moiety for T cell stimulatory function, comprising:

20

providing a culture of human T cell blasts and a Raji cell line;  
contacting the culture with the moiety; and  
assessing cytokine production by the culture.

25

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67. The assay of Claim 66, wherein the T cell blasts are washed prior to culture with the Raji cell line.

30

68. The assay of Claim 66, wherein the cytokine is IL-2.

20

69. The assay of Claim 66, wherein the cytokine is IFN- $\gamma$ .

35

70. The assay of Claim 66, wherein cytokine production is assessed in supernatant isolated from the culture.

40

25

71. The assay of Claim 66, wherein the moiety is an antibody.

72. The assay of Claim 66, wherein the antibody binds to CTLA-4.

45

30

73. A T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a culture comprising human T cell blasts and a Raji cell line with an agent and assessing cytokine production by the culture.

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74. The assay of Claim 73, wherein the T cell blasts are washed prior to culture with the Raji cell line.

10

5 75. The assay of Claim 73, wherein the cytokine is IL-2.

76. The assay of Claim 73, wherein the cytokine is IFN- $\gamma$ .

15

77. The assay of Claim 73, wherein cytokine production is assessed in supernatant isolated from the culture.

10

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78. The assay of Claim 73, wherein the agent is an antibody.

79. The assay of Claim 73, wherein the antibody binds to CTLA-4.

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80. A method for screening an agent for T cell stimulatory activity, comprising:

30

contacting the agent with a cell culture comprising human T cell blasts and a Raji cell line; and

20

assessing cytokine production by the culture.

35

81. The method of Claim 80, wherein the T cell blasts are washed prior to culture with the Raji cell line.

40

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82. The method of Claim 80, wherein the cytokine is IL-2.

83. The method of Claim 80, wherein the cytokine is IFN- $\gamma$ .

45

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84. The method of Claim 80, wherein cytokine production is assessed in supernatant isolated from the culture.

50

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5                   85.    The method of Claim 80, wherein the agent is an antibody that  
binds to CTLA-4.

10                   86.    An assay for measuring T cell stimulation, comprising:  
5                   providing a population of human peripheral blood mononuclear  
cells or human whole blood stimulated with staphylococcus enterotoxin  
A;

15                   contacting the culture with an agent; and  
measuring cytokine production by the cell population.

10                   87.    The assay of Claim 86, wherein the cytokine is IL-2.

20                   88.    The assay of Claim 86, wherein cytokine production is measured  
in supernatant isolated from the cell population.

25                   89.    The assay of Claim 86, wherein the agent is an antibody.

30                   90.    The assay of Claim 86, wherein the antibody binds to CTLA-4.

20                   91.    A functional assay for screening a moiety for T cell stimulatory  
function, comprising:

35                   providing a population of human peripheral blood mononuclear  
cells or human whole blood stimulated with staphylococcus enterotoxin  
A;

40                   25                   contacting the culture with the moiety; and  
assessing cytokine production by the cell population.

45                   92.    The assay of Claim 91, wherein the cytokine is IL-2.

30                   93.    The assay of Claim 91, wherein cytokine production is assessed  
in supernatant isolated from the cell population.

5 94. The assay of Claim 91, wherein the moiety is an antibody .

10 95. The assay of Claim 91, wherein the antibody binds to CTLA-4.

5 96. A T cell stimulatory assay for CTLA-4 inhibitory function,  
comprising contacting a population of human peripheral blood mononuclear  
15 cells or human whole blood stimulated with staphylococcus enterotoxin A with  
an agent and assessing cytokine production by the cell population.

10 97. The assay of Claim 96, wherein the cytokine is IL-2.

20 98. The assay of Claim 96, wherein cytokine production is assessed  
in supernatant isolated from the cell population.

25 99. The assay of Claim 96, wherein the agent is an antibody.

30 100. The assay of Claim 96, wherein the antibody binds to CTLA-4.

35 101. A method for screening an agent for T cell stimulatory activity,  
20 comprising:  
contacting the agent with a population of human peripheral blood  
mononuclear cells or human whole blood stimulated with  
staphylococcus enterotoxin A; and  
35 assessing cytokine production by the cell population.

40 102. The method of Claim 101, wherein the cytokine is IL-2.

45 103. The method of Claim 101, wherein cytokine production is  
assessed in supernatant isolated from the cell population.

30 104. The method of Claim 101, wherein the agent is an antibody that  
50 binds to CTLA-4.



1/48

**Figure 1A****4.1.1 Heavy Chain DNA**

```

ATGGAGTTTG GGCTGAGCTG GGTTCCTC GTTGCTCTT TAAGAGGTGT 50
CCAGTGTCTAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG 100
GGAGGTCCCT GAGACTCTCC TGTGTAGCGT CTGGATTAC CTTCAGTAGC 150
CATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT 200
GGCAGTTATA TGGTATGATG GAAGAAATAA ATACTATGCA GACTCCGTGA 250
AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC GCTGTTTCTG 300
CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG 350
AGGAGGTCAC TTCGGTCCTT TTGACTACTG GGGCCAGGGA ACCCTGGTCA 400
CCGTCTCCTC AGCCTCCACC AAGGGCCCAT CGGTCTTCCC CCTGGCGCCC 450
TGCTCCAGGA GCACCTCCGA GAGCACAGCG GCCCTGGGCT GCCTGGTCAA 500
GGACTACTTC CCCGAACCGG TGACGGGTGC GTGGAAGTCA GGCCTCTGA 550
CCAGCGGCGT GCACACCTTC CCAGCTGTCC TACAGTCCTC AGGACTCTAC 600
TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAACTTCG GCACCCAGAC 650
CTACACCTGC AACGTAGATC ACAAGCCCAG CAACACCAAG GTGGACAAGA 700
CAGTTGAGCG CAAATGTTGT GTCGAGTGCC CACCGTGCCC AGCACCACCT 750
GTGGCAGGAC CGTCAGTCTT CCTCTTCCCC CCAAACCCA AGGACACCTT 800
CATGATCTCC CGGACCCCTG AGGTCACGTG CGTGGTGGTG GACGTGAGCC 850
ACGAAGACCC CGAGGTCCAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG 900
CATAATGCCA AGACAAAGCC ACGGGAGGAG CAGTTCAACA GCACGTTCCG 950
TGTGGTCAGC GTCCTCACCG TTGTGCACCA GGACTGGCTG AACGGCAAGG 1000
AGTACAAGTG CAAGGTCTCC AACAAAGGCC TCCAGCCCC CATCGAGAAA 1050
ACCATCTCCA AAACCAAAGG GCAGCCCCGA GAACACAGG TGTACACCTT 1100
GCCCCATCC CGGGAGGAGA TGACCAAGAA CCAGGTCAGC CTGACCTGCC 1150
TGGTCAAAGG CTTCTACCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT 1200
GGGCAGCCGG AGAACAATA CAAGACCACA CCTCCCATGC TGGACTCCGA 1250
CGGCTCCTTC TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC 1300
AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC 1350
CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA 1392

```

(SEQ ID NO:27)

**4.1.1 Heavy Chain Protein**

```

MEFGLSWVFL VALLRGVQCQ VQLVESGGGV VQPGKSLRLS CVASGFTFSS 50
HGMHWVRQAP GKGLEWVAVI WYDGRNKYYA DSVKGRFTIS RDNSKNTLFL 100
QMNSLRAEDT AVYYCARGGH FGPFDYWGQG TLVTVSSAST KGPSVFPLAP 150
CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 200
SLSSVVTGPS SNFGTQTYTC NVDHKPSNTK VDKTVERKCC VECPPCPAPP 250
VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ FNWYVDGVEV 300
HNAKTKPREE QFNSTFRVVS VLTIVHQDWL NGKEYKCKVS NKGLPAPIEK 350
TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN 400
GQPENNYKTT PPMILDSGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN 450
HYTQKSLSL PGK 463

```

(SEQ ID NO:1)

**Figure 1A** (continued)**4.1.1 Kappa Chain DNA**

```

ATGGAAACCC CAGCGCAGCT TCTCTTCCTC CTGCTACTCT GGCTCCCAGA 50
TACCACCGGA GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT 100
CTCCAGGGGA AAGAGCCACC CTCTCCTGCA GGGCCAGTCA GAGTATTAGC 150
AGCAGCTTCT TAGCCTGGTA CCAGCAGAGA CCTGGCCAGG CTCCCAGGCT 200
CCTCATCTAT GGTGCATCCA GCAGGGCCAC TGGCATCCCA GACAGGTTCA 250
GTGGCAGTGG GTCTGGGACA GACTTCACTC TCACCATCAG CAGACTGGAG 300
CCTGAAGATT TTGCAGTGTA TTA CTGTGTCAG CAGTATGGTA CCTCACCTG 350
GACGTTTCGGC CAAGGGACCA AGGTGGAAAT CAAACGAACT GTGGCTGCAC 400
CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA ATCTGGAAT 450
GCCTCTGTTG TGTGCCTGCT GAATAACTTC TATCCCAGAG AGGCCAAAGT 500
ACAGTGGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC CAGGAGAGTG 550
TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCTG 600
ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT 650
CACCCATCAG GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG 700
AGTGTTAG 708

```

(SEQ ID NO:40)

**4.1.1 Kappa Chain Protein**

```

METPAQLLFL LLLWLPDTTG EIVLTQSPGT LSLSPGERAT LSCRASQSIG 50
SSFLAWYQQR PGQAPRLLIY GASSRATGIP DRFSGSGSGT DFTLTISRLE 100
PEDFAVYYCQ QYGTSPWTFG QGTKVEIKRT VAAPSVFIFP PSDEQLKSGT 150
ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL 200
TLISKADYEH KVIYACEVTHQ GLSSPVTKSF NRGEC 235

```

(SEQ ID NO:14)

3/48

**Figure 1B****4.8.1 Heavy Chain DNA**

```

ATGGAGTTTG GGCTGAGCTG GGTTCCTC GTTGCTCTTT TAAGAGGTGT 50
CCAGTGTCTAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG 100
GGAGGTCCCT GAGACTCTCC TGTACAGCGT CTGGATTAC CTTAGTAAC 150
TATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT 200
GGCAGTTATA TGGTATGATG GAAGTAATAA AACTATGGA GACTCCGTGA 250
AGGGCCGATT CACCATCTCC AGTGACAATT CCAAGAACAC GCTGTATCTG 300
CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG 350
AGGAGAGAGA CTGGGGTCCT ACTTTGACTA CTGGGGCCAG GGAACCCTGG 400
TCACCGTCTC CTCAGCCTCC ACCAAGGGCC CATCGGTCTT CCCCCTGGCG 450
CCCTGCTCCA GGAGCACCTC CGAGAGCACA GCGGCCCTGG GCTGCCTGGT 500
CAAGGACTAC TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCTC 550
TGACCAGCGG CGTGACACAC TTCCCAGCTG TCCTACAGTC CTCAGGACTC 600
TACTCCCTCA GCAGCGTGGT GACCGTGCCC TCCAGCAACT TCGGCACCCA 650
GACCTACACC TGCAACGTAG ATCACAAGCC CAGCAACACC AAGGTGGACA 700
AGACAGTTGA GCGCAAATGT TGTGTGAGT GCCCACCCTG CCCAGCACCA 750
CCTGTGGCAG GACCGTCAGT CTTCTCTTTC CCCCCAAAC CCAAGGACAC 800
CCTCATGATC TCCCGGACCC CTGAGGTCAC GTGCGTGGTG GTGGACGTGA 850
GCCACGAAGA CCCCAGGGTC CAGTTCAACT GGTACGTGGA CGGCGTGGAG 900
GTGCATAATG CCAAGACAAA GCCACGGGAG GAGCAGTTCA ACAGCACGTT 950
CCGTGTGGTC AGCGTCCTCA CCGTTGTGCA CCAGGACTGG CTGAACGGCA 1000
AGGAGTACAA GTGCAAGGTC TCCAACAAAG GCCTCCCAGC CCCCATCGAG 1050
AAAACCATCT CCAAAACCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC 1100
CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC AGCTGACCT 1150
GCCTGGTCAA AGGCTTCTAC CCCAGCGACA TCGCCGTGGA GTGGGAGAGC 1200
AATGGGCAGC CGGAGAACAA CTACAAGACC ACACCTCCCA TGCTGGACTC 1250
CGACGGCTCC TTCTTCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT 1300
GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC 1350
AACCCTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGTA AATGA 1395

```

(SEQ ID NO:28)

**4.8.1 Heavy Chain Protein**

```

MEFGLSWVFL VALLRGVQCQ VQLVESGGGV VQPGSLRLS CTASGFTFSN 50
YGMHWVRQAP GKGLEWVAVI WYDGSNKHYG DSVKGRFTIS SDNSKNTLYL 100
QMNSLRAEDT AVYYCARGER LGSYFDYWQ GTLTVSSAS TKGPSVFPLA 150
PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 200
YSLSSVVTVP SSNFGTQTYT CNVDHKPSNT KVDKTVKRC CVECPCPAP 250
PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE 300
VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE 350
KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES 400
NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH 500
NHYTQKSLSL SPGK 514

```

(SEQ ID NO:2)

4/48

**Figure 1B** (continued)**4.8.1 Kappa Chain DNA**

```

ATGGAAACCC CAGCGCAGCT TCTCTTCCTC CTGCTACTCT GGCTCCCAGA 50
TACCACCGGA GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT 100
CTCCAGGGGA AAGAGCCACC CTCTCCTGCA GGACCAGTGT TAGCAGCAGT 150
TACTTAGCCT GGTACCAGCA GAAACCTGGC CAGGCTCCCA GGCTCCTCAT 200
CTATGGTGCA TCCAGCAGGG CCACTGGCAT CCCAGACAGG TTCAGTGGCA 250
GTGGGTCTGG GACAGACTTC ACTCTACCA TCAGCAGACT GGAGCCTGAA 300
GATTTTGCA TCTATTACTG TCAGCAGTAT GGCATCTCAC CCTTCACTTT 350
CGGCGGAGGG ACCAAGGTGG AGATCAAGCG AACTGTGGCT GCACCATCTG 400
TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG AACTGCCTCT 450
GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG 500
GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTCACAG 550
AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG 600
AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA 650
TCAGGGCCTG AGCTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT 700
AG 702

```

(SEQ ID NO:41)

**4.8.1 Kappa Chain Protein**

```

METPAQLLFL LLLWLPDTTG EIVLTQSPGT LSLSPGERAT LSCRTSVSSS 50
YLAWYQQKPG QAPRLLIYGA SSRATGIPDR FSGSGSGTDF TLTISRLEPE 100
DFAVYYCQY GISPFTFGGG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS 150
VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSTLTTL 200
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC 233

```

(SEQ ID NO:15)

5/48

**Figure 1C****4.14.3 Heavy Chain DNA**

```

CCTGGGAGGT CCCTGAGACT CTCCTGTGCA GCGTCTGGAT TCACCTTCAG 50
TAGTCATGGC ATCCACTGGG TCCGCCAGGC TCCAGGCAAG GGGCTGGAGT 100
GGGTGGCAGT TATATGGTAT GATGGAAGAA ATAAAGACTA TGCAGACTCC 150
GTGAAGGGCC GATTCACCAT CTCCAGAGAC AATTCCAAGA AGACGCTGTA 200
TTTGCAAATG AACAGCCTGA GAGCCGAGGA CACGGCTGTG TATTACTGTG 250
CGAGAGTGGC CCCACTGGGG CCACTTGACT ACTGGGGCCA GGAACCCCTG 300
GTCAACGTCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC 350
GCCCTGCTCC AGGAGCACCT CCGAGAGCAC AGCGGCCCTG GGCTGCCTGG 400
TCAAGGACTA CTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCT 450
CTGACCAGCG GCGTGCACAC CTTCCAGCT GTCCTACAG 489

```

(SEQ ID NO:29)

**4.14.3 Heavy Chain Protein**

```

PGRSLRLSCA ASGFTFSSHG IHWVRQAPGK GLEWVAWIY DGRNKDYADS 50
VKGRFTISRD NSKKTLYLQM NSLRAEDTAV YYCARVAPLG PLDYWGQGT 100
VTVSSASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA 150
LTSGVHTFPA VLQ 163

```

(SEQ ID NO:3)

**4.14.3 Kappa Chain DNA**

```

GGCACCTGT CTTGTCTCC AGGGGAAAGA GCCACCCTCT CCTGCAGGGC 50
CAGTCAGAGT GTCAGCAGCT ACTTAGCCTG GTACCAGCAG AAACCTGGCC 100
AGGCTCCCAG ACTCCTCATC TATGGTGCAT CCAGCAGGGC CACTGGCATC 150
CCAGACAGGT TCAGTGGCAG TGGGTCTGGG ACAGACTTCA CTCTACCAT 200
CAGCAGACTG GAGCCTGAGG ATTTTGCACT GTATTACTGT CAGCAGTATG 250
GTAGGTCACC ATCACTTTC GGCCCTGGGA CCAAAGTGGA TATCAAGCGA 300
ACTGTGGCTG CACCATCTGT CTTTCATCTC CCGCATCTG ATGAGCAGTT 350
GAAATCTGGA ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA 400
GAGAGGCCAA AGTACAG 417

```

(SEQ ID NO:42)

**4.14.3 Kappa Chain Protein**

```

GTLSSLSPGER ATLSCRASQS VSSYLAWYQQ KPGQAPRLLI YGASSRATGI 50
PDRFSGSGSG TDFLTISRLEPEDFAVYYC QQYGRSPFTF GPGTKVDIKR 100
TVAAPSVPFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQ 139

```

(SEQ ID NO:16)

SUBSTITUTE SHEET (RULE 26)

6/48

**Figure 1D****6.1.1 Heavy Chain DNA**

```

ATGGAGTTTG GGCTGAGCTG GGTTCCTC GTTGCTCTT TAAGAGGTGT 50
CCAGTGTCTAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCGAGCCTG 100
GGAGGTCCCT GAGACTCTCC TGTACAGCGT CTGGATTAC CTTCAGTAGT 150
TATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT 200
GGCAGTTATA TGGTATGATG GAAGCAATAA AACTATGCA GACTCCGCGA 250
AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG 300
CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG 350
AGCCGGACTG CTGGGTACTT TTGACTACTG GGGCCAGGGA ACCCTGGTCA 400
CCGTCTCCTC AGCCTCCACC AAGGGCCCAT CGTCTTCCC CCTGGCGCCC 450
TGCTCCAGGA GCACCTCCGA GAGCACAGCG GCCCTGGGCT GCCTGGTCAA 500
GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAC TCA GCGCTCTGA 550
CCAGCGGCGT GCACACCTTC CCAGCTGTCC TACAGTCCTC AGGACTCTAC 600
TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAACTTCG GCACCCAGAC 650
CTACACCTGC AACGTAGATC ACAAGCCCAG CAACACCAAG GTGGACAAGA 700
CAGTTGAGCG CAAATGTTGT GTCGAGTGCC CACCGTGCCC AGCACCACCT 750
GTGGCAGGAC CGTCAGTCTT CCTCTTCCCC CAAAACCCA AGGACACCCT 800
CATGATCTCC CGGACCCCTG AGGTCACGTG CGTGGTGGTG GACGTGAGCC 850
ACGAAGACCC CGAGGTCCAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG 900
CATAATGCCA AGACAAAGCC ACGGGAGGAG CAGTTCAACA GCACGTTCCG 950
TGTGGTCAGC GTCCTCACCG TTGTGCACCA GGACTGGCTG AACGGCAAGG 1000
AGTACAAGTG CAAGGTCTCC AACAAAGGCC TCCCAGCCCC CATCGAGAAA 1050
ACCATCTCCA AAACCAAAGG GCAGCCCCGA GAACACAGG TGTACACCCT 1100
GCCCCATCC CGGGAGGAGA TGACCAAGAA CCAGGTCAGC CTGACCTGCC 1150
TGGTCAAAGG CTTCTACCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT 1200
GGGCAGCCGG AGAACAAC TAAGACCACA CCTCCCATGC TGGACTCCGA 1250
CGGCTCCTTC TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC 1300
AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC 1350
CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA 1392

```

(SEQ ID NO:30)

**6.1.1 Heavy Chain Protein**

```

MEFGLSWVFL VALLRGVQCQ VQLVESGGGV VEPGRSLRLS CTASGFTFSS 50
YGMHWVRQAP GKGLEWVAVI WYDGSNKHYA DSAKGRFTIS RDNSKNTLYL 100
QMNSLRAEDT AVYYCARAGL LGYFDYWQGG TLVTVSSAST KGPSVFPLAP 150
CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY 200
SLSSVVTVPs SNFGTQTYTC NVDHKPSNTK VDKTVERKCC VECPPCPAPP 250
VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ FNWYVDGVEV 300
HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS NKGLPAPIEK 350
TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN 400
GQPENNYKTT PPMLDSGGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN 450
HYTQKSLSLs PGK 463

```

(SEQ ID NO:4)

**Figure 1D** (continued)**6.1.1 Kappa Chain DNA**

```

ATGGAACCC CAGCGCAGCT TCTCTTCCTC CTGCTACTCT GGCTCCCAGA 50
TACCACCGGA GAAATTGTGT TGACGCAGTC TCCAGGCACC CTGTCTTTGT 100
CTCCAGGGGA AAGAGCCACC CTCTCCTGTA GGGCCAGTCA AAGTGTTAGC 150
AGCTACTTAG CCTGGTACCA ACAGAAACCT GGCCAGGCTC CCAGGCCCT 200
CATCTATGGT GTATCCAGCA GGGCCACTGG CATCCCAGAC AGGTTTCAGTG 250
GCAGTGGGTC TGGGACAGAC TTCACTCTCA CCATCAGCAG ACTGGAGCCT 300
GAAGATTTTG CAGTGTATTA CTGTCAGCAG TATGGTATCT CACCATTAC 350
TTTCGGCCCT GGGACCAAAG TGGATATCAA ACGAACTGTG GCTGCACCAT 400
CTGTCTTCAT CTTCGCCCA TCTGATGAGC AGTTGAAATC TGGAAGTGCC 450
TCTGTTGTGT GCCTGCTGAA TAACTTCTAT CCCAGAGAGG CCAAAGTACA 500
GTGGAAGGTG GATAACGCCC TCCAATCGGG TAACTCCCAG GAGAGTGTCA 550
CAGAGCAGGA CAGCAAGGAC AGCACCTACA GCCTCAGCAG CACCCTGACG 600
CTGAGCAAAG CAGACTACGA GAAACACAAA GTCTACGCCT GCGAAGTCAC 650
CCATCAGGGC CTGAGCTCGC CCGTCACAAA GAGCTTCAAC AGGGGAGAGT 700
GTTAG 705

```

(SEQ ID NO:43)

**6.1.1 Kappa Chain Protein**

```

METPAQLLFL LLLWLPDTTG EIVLTQSPGT LSLSPGERAT LSCRASQSVS 50
SYLAWYQQKP GOAPRPLIYG VSSRATGIPD RFSGSGSGTD FTLTISRLEP 100
EDFAVYYCQQ YGISPFTFGP GTKVDIKRTV AAPSVFIFPP SDEQLKSGTA 150
SVVCLLNIFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSLT 200
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK 234

```

(SEQ ID NO:17)

8/48

**Figure 1E****3.1.1 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG 50
ATTACACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TAATAAATAC 150
TATGCAGACT CCGTGAAGGG CCGATTACAC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCAGAGAGG GCCCGTATAA TAACCCCTTG TATGGACGTC 300
TGGGGCCCAAG GGACCACGGT CACCGTCTCC TCAGCCTCCA CCAAGGGCCC 350
ATCGGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG 400
CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG 450
TCGTGGAAC T CAGGCGCTCT GACCAGCGGC GTGCACACCT TCCCAGCTGT 500
CCTACAG 507

```

(SEQ ID NO:31)

**3.1.1 Heavy Chain Protein**

```

GVVQPGRSRLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSNKY 50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARG ARIITPCMDV 100
WGQGTITVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV 150
SWNSGALTSG VHTFPAVLQ 169

```

(SEQ ID NO:5)

**3.1.1 Kappa Chain DNA**

```

CAGTCTCCAT CCTCCCTGTC TGCATCTGTA GGAGACAGAG TCACCATCAC 50
TTGCCGGGCA AGTCAGAGCA TTAACACCTA TTAAATTTGG TATCAGCAGA 100
AACCAGGGAA AGCCCCTAAC TTCCTGATCT CTGCTACATC CATTTTGCAA 150
AGTGGGGTCC CATCAAGGTT CCGTGGCAGT GGCTCTGGGA CAAATTTTAC 200
TCTCACCATC AACAGTCTTC ATCCTGAAGA TTTTGCAACT TACTACTGTC 250
AACAGAGTTA CAGTACCCCA TTCACTTTTCG GCCCTGGGAC CAAAGTGGAT 300
ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA 350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT 400
TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA 450
TCGGGTAA 458

```

(SEQ ID NO:44)

**3.1.1 Kappa Chain Protein**

```

QSPSSLSASV GDRVITTCRA QSINTYLIW YQKPGKAPN FLISATSILQ 50
SGVPSRFRGS GSGTNFTLTI NSLHPEDFAT YYCQSYSTP FTFGPGTKVD 100
IKRTVAAPSV FIFPPSDEQL KSGTASVCL LNNFYPREAK VQWKVDNALQ 150
SG 152

```

(SEQ ID NO:18)



9/48

**Figure 1F****4.10.2 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG TAGCGTCTGG 50
ATTCATCTTC AGTAGTCATG GCATCCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG AAATAAAGAC 150
TATGCAGACT CCGTGAAGGG CCGATTCAAC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATTTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCGAGAGTG GCCCCTACTG GGCCACTTGA CTACTGGGGC 300
CAGGGAACCC TGGTCACCGT CTCCTCAGCC TCCACCAAGG GCCCATCGGT 350
CTTCCCCCTG GCGCCCTGCT CCAGGAGCAC CTCCGAGAGC ACAGCGGCCC 400
TGGGCTGCCT GGTCAAGGAC TACTTCCCCG AACCGGTGAC GGTGTCGTGG 450
AACTCAGGCG CTCTGACCAG CGGCGTGCAC ACCTTCCCAG CTGTCCTACA 500
G 501

```

(SEQ ID NO:32)

**4.10.2 Heavy Chain Protein**

```

GVVQPGRSLR LSCVASGFIF SSHGIHWVRQ APGKGLEWVA VIWYDGRNKD 50
YADSVKGRFT ISRDN SKNTL YLQMNSLRAE DTA VYYCARV APLGPLDYWG 100
QGT LVTVSSA STKGPSVFPL APCSRSTSES TAALGCLVKD YFPEPVTVSW 150
NSGALTSGVH TFP AVLQ 167

```

(SEQ ID NO:6)

**4.10.2 Kappa Chain DNA**

```

TCTCCAGGCA CCTGTCTTT GTCTCCAGGG GAAAGAGCCA CCCTCTCCTG 50
CAGGGCCAGT CAGAGTATTA GCAGCAATTT CTTAGCCTGG TACCAGCAGA 100
AACCTGGCCA GGCTCCCAGG CTCCTCATCT ATCGTCCATC CAGCAGGGCC 150
ACTGGCATCC CAGACAGTTT CAGTGGCAGT GGGTCTGGGA CAGACTTCAC 200
TCTCACCATC AGCAGACTGG AGCCTGAGGA TTTTGCATTA TATTACTGTC 250
AGCAGTATGG TACGTCACCA TTCACTTTCG GCCCTGGGAC CAAAGTGGAT 300
ATCAAGCGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA 350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT 400
TCTATCCCAG AGAGGCCAAA GTACAG 426

```

(SEQ ID NO:45)

**4.10.2 Kappa Chain Protein**

```

SPGTLSPG ERATLSCRAS QSISSNFLAW YQKPGQAPR LLIYRPSSRA 50
TGIPDSFSGS GSGTDFTLTI SRLEPEDFAL YYCQYGTSP FTFGPGTKVD 100
IKRTVAAPSV FIFPPSDEQL KSGTASVCL LNNFYPREAK VQ 142

```

(SEQ ID NO:19)

10/48

**Figure 1G****2.1.3 Heavy Chain DNA**

```

TCGGGCCCCAG GACTGGTGAA GCCTTCACAG ATCCTGTCCC TCACCTGCAC 50
TGTCTCTGGT GGCTCCATCA GCAGTGGTGG TCACTACTGG AGCTGGATCC 100
GCCAGCACCC AGGGAAGGGC CTGGAGTGA TTGGGTACAT CTATTACATT 150
GGGAACACCT ACTACAACCC GTCCCTCAAG AGTCGAGTTA CCATATCAGT 200
AGACACGTCT AAGAACCAGT TCTCCCTGAA GCTGAGCTCT GTGACTGCCG 250
CGGACACGGC CGTGTATTAT TGTGCGAGAG ATAGTGGGGA CTACTACGGT 300
ATAGACGTCT GGGGCCAAGG GACCACGGTC ACCGTCTCCT CAGCTTCCAC 350
CAAGGGCCCCA TCCGTCTTCC CCCTGGCGCC CTGCTCCAGG AGCACCTCCG 400
AGAGCACAGC CGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCCGAACCG 450
GTGACGGTGT CGTGGAATC AGGCGCCCTG ACCAGCGGCG TGCACACCTT 500
CCCGGCTGTC CTACAA 516

```

(SEQ ID NO:33)

**2.1.3 Heavy Chain Protein**

```

SGPGLVKPSQ ILSLTCTVSG GSISGGHYW SWIRQHPGKG LEWIGYIYYI 50
GNTYYNPSLK SRVTISVDT S KNQFSLXLSS VTAADTAVYY CARDSGDYVG 100
IDVWGQGTTV TVSSASTKGP SVFPLAPCSR STSESTAALG CLVKDYFPEP 150
VTVSWNSGAL TSGVHTFPAV LQ 172

```

(SEQ ID NO:7)

**2.1.3 Kappa Chain DNA**

```

TCTCCAGACT TTCAGTCTGT GACTCCAAAG GAGAAAGTCA CCATCACCTG 50
CCGGGCCAGT CAGAGCATTG GTAGTAGCTT ACATTGGTAT CAGCAGAAAC 100
CAGATCAGTC TCCAAAGCTC CTCATCAAGT ATGCTTCCCA GTCCTTCTCT 150
GGGGTCCCCT CGAGGTTTCA TGGCAGTGA TCTGGGACAG ATTTACCCCT 200
CACCATCAAT AGCCTGGAAG CTGAAGATGC TGCAACGTAT TACTGTCATC 250
AGAGTAGTAG TTTACCGCTC ACTTTCGGCG GAGGGACCAA GGTGGAGATC 300
AAACGAACTG TGGCTGCACC ATCTGTCTT ATCTTCCCGC CATCTGATGA 350
GCAGTTGAAA TCTGGAATG CCTCTGTTGT GTGCCTGCTG AATAACTTCT 400
ATCCCAGAGA GGCCAAAGTA CAGTGAAGG TGGATAACGC CCTCCAATCG 450
GGTAACTCCC AGGAG 465

```

(SEQ ID NO:46)

**2.1.3 Kappa Chain Protein**

```

SPDFQSVTPK EKVITICRAS QSIGSSLHWY QQKPDQSPKL LIKYASQSFS 50
GVPSRFSGSG SGTDFTLTIN SLEAEDAATY YCHQSSSLPL TFGGGTKVEI 100
KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS 150
GNSQE 155

```

(SEQ ID NO:20)

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11/48

**Figure 1H****4.13.1 Heavy Chain DNA**

```

CCTGGGAGGT CCCTGAGACT CTCCTGTGCA GCGTCTGGAT TCACCTTCAG 50
TAGTCATGGC ATCCACTGGG TCCGCCAGGC TCCAGGCAAG GGGCTGGAGT 100
GGGTGGCAGT TATATGGTAT GATGGAAGAA ATAAAGACTA TGCAGACTCC 150
GTGAAGGGCC GATTCAACAT CTCCAGAGAC AATTCCAAGA ACACGCTGTA 200
TTTGCAAATG AACAGCCTGA GAGCCGAGGA CACGGCTGTG TATTACTGTG 250
CGAGAGTGGC CCCACTGGGG CCACTTGACT ACTGGGGCCA GGGAAACCTG 300
GTCACCGTCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC 350
GCCCTGCTCC AGGAGCACCT CCGAGAGCAC AGCGGCCCTG GGCTGCCTGG 400
TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCT 450
CTGACCAGC                                     459

```

(SEQ ID NO:34)

**4.13.1 Heavy Chain Protein**

```

PGRSLRLSCA ASGFTFSSHG IHWVRQAPGK GLEWVAVIWY DGRNKDYADS 50
VKGRFTISR D NSKNTLYLQM NSLRAEDTAV YYCARVAPLG PLDYWGQGT 100
VTSSASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA 150
LTS                                     153

```

(SEQ ID NO:8)

**4.13.1 Kappa Chain DNA**

```

CAGTCTCCAG GCACCCTGTC TTTGTCTCCA GGGGAAAGAG CCACCCTCTC 50
CTGCAGGGCC AGTCAGAGTG TCAGCAGCTA CTTAGCCTGG TACCAGCAGA 100
AACCTGGCCA GGCTCCCAGG CTCCTCATCT ATGGTGCATC CAGCAGGGCC 150
ACTGGCATCC CAGACAGGTT CAGTGGCAGT GGGTCTGGGA CAGACTTCAC 200
TCTCACCATC AGCAGACTGG AGCCTGAGGA TTTTGCACTG TATTACTGTC 250
AACAGTATGG TAGGTCACCA TTCACTTTCG GCCCTGGGAC CAAAGTAGAT 300
ATCAAGCGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA 350
TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT 400
TCTATCCCAG AGAGGCCAAA GTACAGTGGA AAGGTGGATA 440

```

(SEQ ID NO:47)

**4.13.1 Kappa Chain Protein**

```

QSPGTLSP GERATLSCRA SQSVSSYLAW YQQKPGQAPR LLIYGASSRA 50
TGIPDRFSGS GSGTDFTLTI SRLEPEDFAV YYCQYGRSP FTFGPGTKVD 100
IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKGG 146

```

(SEQ ID NO:21)

12/48

**Figure 11****11.2.1 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG 50
ATTCACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TAATAAATAC 150
TATGCAGACT CCGTGAAGGG CCGATTCAAC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCAGAGAT CCGAGGGGAG CTACCCTTTA CTACTACTAC 300
TACCGGTKGG ACGTCTGGGG CCAAGGGACC ACGGTCACCG TCTCCTCAGC 350
CTCCACCAAG GGCCCATCGG TCTTCCCCCT GGCGCCCTGC TCCAGGAGCA 400
CTCCCGAGAG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA CTAATTCCCC 450
GAACCGGTGA CCGTGTCTGT GAATCAGGC GCTCTGACCA GCGGCGTGCA 500
CAC 503

```

(SEQ ID NO:35)

**11.2.1 Heavy Chain Protein**

```

GVVQPGRSLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSNKY 50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARD PRGATLYYYY 100
YRXDVWGQGT TVTVSSASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP 150
EPVTVSWNSG ALTSGVH

```

(SEQ ID NO:9)

**11.2.1 Kappa Chain DNA**

```

CCATCCTCCC TGTCTGCATC TGTAGGAGAC AGAGTCACCA TCACTTGCCG 50
GGCAAGTCAG AGCATTAAACA GCTATTTAGA TTGGTATCAG CAGAAACCAG 100
GGAAAGCCCC TAAACTCCTG ATCTATGCTG CATCCAGTTT GCAAAGTGGG 150
GTCCCATCAA GGTTCAGTGG CAGTGGATCT GGGACAGATT TCACTCTCAC 200
CATCAGCAGT CTGCAACCTG AAGATTTTGC AACTTACTAC TGTCACAGT 250
ATTACAGTAC TCCATTCACT TTCGGCCCTG GGACCAAAGT GGAAATCAAA 300
CGAACTGTGG CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA 350
GTTGAAATCT GGAAGTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC 400
CCAGAGAGGC CAAAGTA 417

```

(SEQ ID NO:48)

**11.2.1 Kappa Chain Protein**

```

PSSLSASVGD RVTITCRASQ SINSYLDWYQ QKPGKAPKLL IYAASSLQSG 50
VPSRFGSGS GTDFTLTISS LQPEDFATYY CQYYSTPFT FGPGTKVEIK 100
RTVAAPSVFI FPPSDEQLKS GTASVCLLN NFYPREAKV 139

```

(SEQ ID NO:22)

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13/48

**Figure 1J****11.6.1 Heavy Chain DNA**

```

GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCGTCTGG 50
ATTACCTTC AGTAGCTATG GCATGCACTG GGTCCGCCAG GCTCCAGGCA 100
AGGGGCTGGA GTGGGTGGCA GTTATATGGT ATGATGGAAG TCATAAATAC 150
TATGCAGACT CCGTGAAGGG CCGATTCCACC ATCTCCAGAG ACAATTCCAA 200
GAACACGCTG TATCTGCAAA TGAACAGCCT GAGAGCCGAG GACACGGCTG 250
TGTATTACTG TGCAGAGAGC GCTGTAGTAG TACCAGCTGC TATGGACGTC 300
TGGGGCCAAG GGACCACGGT CACCGTCTCC TCAGCCTCCA CCAAGGGCCC 350
ATCGTCTTC CCCCTGGCGC CCTGCTCCAG GAGCACCTCC GAGAGCACAG 400
CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG 450
T 451

```

(SEQ ID NO:36)

**11.6.1 Heavy Chain Protein**

```

GVVQPGRSLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGS HKY 50
YADSVKGRFT ISRDNSKNTL YLQMNSLRAE DTAVYYCARG AVVVPAA MDV 100
WGQGTITVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEP VTV 150
S 151

```

(SEQ ID NO:10)

**11.6.1 Kappa Chain DNA**

```

ACCCAGTCTC CATCTCCCT GTCTGCATCT GTAGGAGACA GAGTCACCAT 50
CACTTGCCGG GCAAGTCAGA ACATTAGCAG GTATTTAAAT TGGTATCAAC 100
AGAAACCAGG GAAAGCCCCT AAGTTCCTGA TCTATGTTGC ATCTATTTTG 150
CAAAGTGGGG TCCCATCAGG GTTCAGTGCC AGTGGATCTG GGCCAGATTT 200
CACTCTNACC ATCAGCAGTC TGCAACCTGA AGATTTTGCA ACTTACTACT 250
GTCAACAGAG TTACAGTACC CCATTCATT TCGGCCCTGG GACCAAAGTG 300
GATATCAAAC GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC 350
TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA 400
AC 402

```

(SEQ ID NO:49)

**11.6.1 Kappa Chain Protein**

```

TQSPSSLSAS VGDRVITICR ASQNISRYLN WYQQKPGKAP KFLIYVASIL 50
QSGVPSGFSF SGSGPDFTLT ISSLPEDFA TYQCQSYST PFTFGPGTKV 100
DIKRTVAAPS VFIFPPSDEQ LKSGTASVVC LLNN 134

```

(SEQ ID NO:23)

SUBSTITUTE SHEET (RULE 26)

14/48

**Figure 1K****11.7.1 Heavy Chain DNA**

```

GTGGTCCAGC CTGGGAGGTC CCTGAGACTC TCCTGTGCAG CGTCTGGATT 50
CACCTTCAGT AGCNGTGGCA TGCACTGGGT CCGCCAGGCT CCAGGCAAGG 100
GGCTGGAGTG GGTGGCAGTT ATATGGTCTG ATGGAAGTCA TAAATACTAT 150
GCAGACTCCG TGAAGGGCCG ATTCACCATC TCCAGAGACA ATTCCAAGAA 200
CACGCTGTAT CTGCAAATGA ACAGCCTGAG AGCCGAGGAC ACGGCTGTGT 250
ATTACTGTGC GAGAGGAACT ATGATAGTAG TGGGTACCCT TGACTIONTGG 300
GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC 350
GGTCTTCCCC CTGGCGCCCT GCTCCAGGAG CACCTCCGAG AGCACAGCGG 400
CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCG 438

```

(SEQ ID NO:37)

**11.7.1 Heavy Chain Protein**

```

VVQPGRSLRL SCAASGFTFS SXGMHWVRQA PGKGLEWVAV IWSDGSHKYY 50
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVVYCARGT MIVVGTLDYW 100
QGQTLVTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEP 146

```

(SEQ ID NO:11)

**11.7.1 Kappa Chain DNA**

```

ACCCAGTCTC CATCCTCCCT GTCTGCATCT GTAGGAGACA GAGTCACCAT 50
CACTTGCCGG GCAAGTCAGA GCATTGCAA CTATTTAAAT TGGTATCAGC 100
AGAAACCAGG AAAAGCCCCT AGGGTCCTGA TCTATGCTGC ATCCAGTTTG 150
CAAGGTGGGG TCCCGTCAAG GTTCAGTGGC AGTGGATCTG GGACAGATTG 200
CACTCTCACC ATCAGCAGTC TGCAACCTGA AGATTTTGCA ACTTACTACT 250
GTCAACAGAG TTAACTACC CCATTCACTT TCGGCCCTGG GACCAGAGTG 300
GATATCGAAC GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC 350
TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTGTGTGTC CTGCTGAATA 400
ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCTAT 450
T 451

```

(SEQ ID NO:50)

**11.7.1 Kappa Chain Protein**

```

TQSPSSLSAS VGDRVITICR ASQICNYLN WYQQKPGKAP RVLIYAASSL 50
QGGVPSRFSG SGSGIDCTLT ISSLQPEDFA TYQCQSYIT PFTFGPGTRV 100
DIERTVAAPS VFIFPPSDEQ LKSGTASVVC LLNMFYPREA KVQWKVDNAY 150

```

(SEQ ID NO:24)

15/48

**Figure 1L****12.3.1 Heavy Chain DNA**

TCCTGTGCAG CGTCTGGATT CACCTTCAGT TACTATGGCG

```

TCTGGGGGAG GCGTGGTCCA GCCTGGGAGG TCCCTGAGAC TCTCCTGTGC 50
AGCGTCTGGA TTCACCTTCA GTAGCTATGG CGTGCACTGG GTCCGCCAGG 100
CTCCAGGCAA GGGGCTGGAG TGGGTGGCAG TTATATGGTA TGATGGAAGT 150
AATAAATACT ATGCAGACTC CGTGAAGGGC CGATTCACCA TCTCCAGAGA 200
CAATTCCAAG AGCACGCTGT ATCTGCAAAT GAACAGCCTG AGAGCCGAGG 250
ACACGGCTGT GTATTATTGT GCGAGAGACT CGTATTACGA TTTTGGAGT 300
GGTCGGGGCG GTATGGACGT CTGGGGCCAA GGGACCACGG TCACCGTCTC 350
CTCAGCCTCC ACCAAGGGCC CATCGGTCTT CCCCCTGGCG CCCTGCTCCA 400
GGAGCACCTC CGAGAGCACA GCGGCCCTGG GCTGCCTGGT CAAGGACTAC 450
TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCTC TGACCAGCGG 500
CGTGCACACC TTCCCAGCTG TC

```

(SEQ ID NO:38)

**12.3.1.1 Heavy Chain Protein**

```

SGGGVVQPGR SLRLSCAASG FTFSSYGVHW VRQAPGKGLE WVAVIWYDGS 50
NKYYADSVKG RFTISRDN SK STLYLQMN SL RAEDTAVYYC ARDSYYDFWS 100
GRGMDVWGQ GTTVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY 150
FPEPVTVSWN SGALTSGVHT FPAV

```

(SEQ ID NO:12)

**12.3.1.1 Kappa Chain DNA**

```

CCACTCTCCC TGCCCGTCAC CCTTGGACAG CCGGCCTCCA TCTCCTGCAG 50
GTCTAGTCAA AGCCTCGTAT ACAGTGATGG AAACACCTAC TTGAATTGGT 100
TTCAGCAGAG GCCAGGCCAA TCTCCAAGGC GCCTAATTTA TAAGGTTTCT 150
AACTGGGACT CTGGGGTCCC AGACAGATTC AGCGGCAGTG GGTCAGGCAC 200
TGATTTTACA CTGAAAATCA GCAGGGTGGA GGCTGAGGAT GTTGGGGTTT 250
ATTACTGCAT GCAAGGTTCA CACTGGCCTC CGACGTTCCG CCAAGGGACC 300
AAGGTGGAAT TCAAACGAAC TGTGGCTGCA CCATCTGTCT TCATCTTCCC 350
GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCCTCTGTT GTGTGCCTGC 400
TGAATAACTT CTATCCAC

```

(SEQ ID NO:51)

**12.3.1.1 Kappa Chain Protein**

```

PLSLPVTLGQ PASISCRSSQ SLVYSDGNTY LNWFAQRPQ SPRLIYKVS 50
NWDSGVPDRF SGSGSGTDF LKISRVEAED VGVYYCMQGS HWPPTFGQGT 100
KVEIKRTVAA PSVFIFPPSD EQLKSGTASV VCLLNPFYP

```

(SEQ ID NO:25)

16/48

**Figure 1M****12.9.1.1 Heavy Chain DNA**

```

GTCCAGCCTG GGAGGTCCCT GAGACTCTCC TGTGCAGCGT CTGGATTAC 50
CTTCAGTAAC TATGCCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC 100
TGGAGTGGGT GGTAGTTATT TGGCATGATG GAAATAATAA ATACTATGCA 150
GAGTCCGTGA AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC 200
GCTGTATCTG CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTATATT 250
ACTGTGCGAG AGATCAGGGC ACTGGCTGGT ACGGAGGCTT TGACTTCTGG 300
GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GCCTCCACCA AGGGCCCATC 350
GGTCTTCCCC CTGGCGCCCT GCTCCAGGAG CACCTCCGAG AGCACAGCGG 400
CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCTG 450
TGGAACTCAG GCGCTCTGAC CAGCGGCGTG CACACCTTCC 490

```

(SEQ ID NO:39)

**12.9.1.1 Heavy Chain Protein**

```

VQPGRLRLS CAASGFTFSN YAMHWVRQAP GKGLEWVVI WHDGNNKYIA 50
ESVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARDQG TGWYGGFDFW 100
GQGTILVTSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS 150
WNSGALTSGV HTF 163

```

(SEQ ID NO:13)

**12.9.1.1 Kappa Chain DNA**

```

CCTGGAGAGC CGGCTTCCAT CTCTGCAGG TCTAGTCAGA GCCTCCTGCA 50
TAGTAATGGA TACAACTATT TGGATTGGTA CCTGCAGAAG CCAGGACAGT 100
CTCCACAGCT CCTGATCTAT TTGGGTTCTA ATCGGGCCTC CGGGGTCCCT 150
GACAGGTTCA GTGGCAGTGG ATCAGGCACA GATTTTACAC TGAAACTCAG 200
CAGAGTGGAG GCTGAGGATG TTGGGGTTTA TTAGTGCATG CAAGCTCTAC 250
AAACTCCTCT CACTTTCGGC GGAGGGACCA AGGTGGAGAT CAAACGAACT 300
GTGGCTGCAC CATCTGTCTT CATCTTCCCG CCATCTGATG AGCAGTTGAA 350
ATCTGGAAC TGCCTCTGTTG TGTGCCTGCT GAATAACTTC TATCCCAGAR 400
AGGCCAAAGT ACATTCCAT 419

```

(SEQ ID NO:52)

**12.9.1.1 Kappa Chain Protein**

```

PGEPAISCR SSQSLLHSNG YNYLDWYLQK PGQSPQLLIY LGSNRASGVP 50
DRFSGSGSGT DFTLKLRSVE AEDVGVIYCM QALQPLTFG GGTKVEIKRT 100
VAAPSVFIFP PSDEQLKSGT ASVVCLLNNF YPR 133

```

(SEQ ID NO:26)

SUBSTITUTE SHEET (RULE 26)



**Figure 2**

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
	G	G	G	G	G			G	G	G		G	
	V	V	V	V	V			V	V	V	V	V	
	V	V	V	V	V			V	V	V	V	V	V
	Q	Q	Q	Q	Q			E	Q	Q	Q	Q	Q
	P	P	P	P	P	P	P	P	P	P	P	P	P
	G	G	G	G	G	G	G	G	G	G	G	G	G
	R	R	R	R	R	R	R	R	R	R	R	R	R
	S	S	S	S	S	S	S	S	S	S	S	S	S
	L	L	L	L	L	L	L	L	L	L	L	L	L
	R	R	R	R	R	R	R	R	R	R	R	R	R
	L	L	L	L	L	L	L	L	L	L	L	L	L
	S	S	S	S	S	S	S	S	S	S	S	S	S
	C	C	C	C	C	C	C	C	C	C	C	C	C
	A	A	V	T	V	A	A	T	A	A	A	A	A
	A	A	A	A	A	A	A	A	A	A	A	A	A
	S	S	S	S	S	S	S	S	S	S	S	S	S
	G	G	G	G	G	G	G	G	G	G	G	G	G
	F	F	F	F	F	F	F	F	F	F	F	F	F
	T	T	T	T	I	T	T	T	T	T	T	T	T
	F	F	F	F	F	F	F	F	F	F	F	F	F
CDR 1	S	S	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	N	S	S	S	S	S	S	S	S	N
	Y	Y	H	Y	H	H	H	Y	Y	Y	C	Y	Y
	G	G	G	G	G	G	G	G	G	G	G	G	A
	M	M	M	M	I	I	I	M	M	M	M	V	M
	H	H	H	H	H	H	H	H	H	H	H	H	H
	W	W	W	W	W	W	W	W	W	W	W	W	W
	V	V	V	V	V	V	V	V	V	V	V	V	V
	R	R	R	R	R	R	R	R	R	R	R	R	R
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	A	A	A	A	A	A	A	A	A	A	A	A	A
	P	P	P	P	P	P	P	P	P	P	P	P	P
	G	G	G	G	G	G	G	G	G	G	G	G	G
	K	K	K	K	K	K	K	K	K	K	K	K	K
	G	G	G	G	G	G	G	G	G	G	G	G	G
	L	L	L	L	L	L	L	L	L	L	L	L	L
	E	E	E	E	E	E	E	E	E	E	E	E	E
	W	W	W	W	W	W	W	W	W	W	W	W	W
	V	V	V	V	V	V	V	V	V	V	V	V	V
	A	A	A	A	A	A	A	A	A	A	A	A	V
	V	V	V	V	V	V	V	V	V	V	V	V	V
	I	I	I	I	I	I	I	I	I	I	I	I	I
	W	W	W	W	W	W	W	W	W	W	W	W	W
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	S	Y	H
	D	D	D	D	D	D	D	D	D	D	D	D	D
	G	G	G	G	G	G	G	G	G	G	G	G	G
CDR	S	S	R	S	R	R	R	S	S	S	S	S	N

**Figure 2** (continued)

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
2													
	N	N	N	N	N	N	N	N	N	H	H	N	N
	K	K	K	K	K	K	K	K	K	K	K	K	K
	Y	Y	Y	H	D	D	D	H	Y	Y	Y	Y	Y
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	A	A	A	G	A	A	A	A	A	A	A	A	A
	D	D	D	D	D	D	D	D	D	D	D	D	E
	S	S	S	S	S	S	S	S	S	S	S	S	S
	V	V	V	V	V	V	V	A	V	V	V	V	V
	K	K	K	K	K	K	K	K	K	K	K	K	K
	G	G	G	G	G	G	G	G	G	G	G	G	G
	R	R	R	R	R	R	R	R	R	R	R	R	R
	F	F	F	F	F	F	F	F	F	F	F	F	F
	T	T	T	T	T	T	T	T	T	T	T	T	T
	I	I	I	I	I	I	I	I	I	I	I	I	I
	S	S	S	S	S	S	S	S	S	S	S	S	S
	R	R	R	S	R	R	R	R	R	R	R	R	R
	D	D	D	D	D	D	D	D	D	D	D	D	D
	N	N	N	N	N	N	N	N	N	N	N	N	N
	S	S	S	S	S	S	S	S	S	S	S	S	S
	K	K	K	K	K	K	K	K	K	K	K	K	K
	N	N	N	N	N	N	K	N	N	N	N	S	N
	T	T	T	T	T	T	T	T	T	T	T	T	T
	L	L	L	L	L	L	L	L	L	L	L	L	L
	Y	Y	F	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	L	L	L	L	L	L	L	L	L	L	L	L	L
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	M	M	M	M	M	M	M	M	M	M	M	M	M
	N	N	N	N	N	N	N	N	N	N	N	N	N
	S	S	S	S	S	S	S	S	S	S	S	S	S
	L	L	L	L	L	L	L	L	L	L	L	L	L
	R	R	R	R	R	R	R	R	R	R	R	R	R
	A	A	A	A	A	A	A	A	A	A	A	A	A
	E	E	E	E	E	E	E	E	E	E	E	E	E
	D	D	D	D	D	D	D	D	D	D	D	D	D
	T	T	T	T	T	T	T	T	T	T	T	T	T
	A	A	A	A	A	A	A	A	A	A	A	A	A
	V	V	V	V	V	V	V	V	V	V	V	V	V
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	C	C	C	C	C	C	C	C	C	C	C	C	C
	A	A	A	A	A	A	A	A	A	A	A	A	A
	R	R	R	R	R	R	R	R	R	R	R	R	R
		G	G	G	V	V	V	A	D	G	G	D	D
		A	G	E	A	A	A	G	P	A	T	S	Q
		R	H	R	P	P	P	L	R	V	M	Y	G
		I	F	L	L	L	L	L	G	V	I	Y	T
CDR 3		I	G	G	G	G	G	G	A	V	V	D	G

**Figure 2** (continued)

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
		T	P	S	P	P	P	Y	T	P	V	F	W
		P	F	Y	L	L	L	F	L	A	G	W	Y
		C	D	F	D	D	D	D	Y	A	T	S	G
		M	Y	D	Y	Y	Y	Y	Y	M	L	G	G
		D	W	Y	W	W	W	W	Y	D	D	R	F
		V	G	W	G	G	G	G	Y	V	Y	G	D
		W	Q	G	Q	Q	Q	Q	Y	W	W	G	F
		G	G	Q	G	G	G	G	G	G	G	M	W
		Q	T	G	T	T	T	T	M	Q	Q	D	G
		G	L	T	L	L	L	L	D	G	G	V	Q
		T	V	L	V	V	V	V	V	T	T	W	G
		T	T	V	T	T	T	T	W	T	L	G	T
		V	V	T	V	V	V	V	G	V	V	Q	L
		T	S	V	S	S	S	S	Q	T	T	G	V
		V	S	S	S	S	S	S	G	V	V	T	T
		S	A	S	A	A	A	A	T	S	S	T	V
		S	S	A	S	S	S	S	T	S	S	V	S
		A	T	S	T	T	T	T	V	A	A	T	S
		S	K	T	K	K	K	K	T	S	S	V	A
		T	G	K	G	G	G	G	V	T	T	S	S
		K	P	G	P	P	P	P	S	K	K	S	T
		G	S	P	S	S	S	S	S	G	G	A	K
		P	V	S	V	V	V	V	A	P	P	S	G
		S	F	V	F	F	F	F	S	S	S	T	P
		V	P	F	P	P	P	P	T	V	V	K	S
		F	L	P	L	L	L	L	K	F	F	G	V
		P	A	L	A	A	A	A	G	P	P	P	F
		L	P	A	P	P	P	P	P	L	L	S	P
		A	C	P	C	C	C	C	S	A	A	V	L
		P	S	C	S	S	S	S	V	P	P	F	A
		C	R	S	R	R	R	R	F	C	C	P	P
		S	S	R	S	S	S	S	P	S	S	L	C
		R	T	S	T	T	T	T	L	R	R	A	S
		S	S	T	S	S	S	S	A	S	S	P	R
		T	E	S	E	E	E	E	P	T	T	C	S
		S	S	E	S	S	S	S	C	S	S	S	T
		E	T	S	T	T	T	T	S	E	E	R	S
		S	A	T	A	A	A	A	R	S	S	S	E
		T	A	A	A	A	A	A	S	T	T	T	S
		A	L	A	L	L	L	L	T	A	A	S	T
		A	G	L	G	G	G	G	S	A	A	E	A
		L	C	G	C	C	C	C	E	L	L	S	A
		G	L	C	L	L	L	L	S	G	G	T	L
		C	V	L	V	V	V		T	C	C	A	G
		L	K	V	K	K	K		A	L	L	A	C
		V	D	K	D	D	D		A	V	V	L	L
		K	Y	D	Y	Y	Y		L	K	K	G	V
		D	F	Y	F	F	F		G	D	D	C	K

**Figure 2** (continued)

CDR	DP5 0	3.1.1	4.1.1	4.8.1	4.10. 2	4.13. 1	4.14. 3	6.1.1	11.2. 1	11.6. 1	11.7. 1	12.3. 1.1	12.9. 1.1
		Y	P	F	P	P	P		C	Y	Y	L	D
		F	E	P	E	E	E		L	F	F	V	Y
		P	P	E	P	P	P		V	P	P	K	F
		E	V	P	V	V	V		K	E	E	D	P
		P	T	V	T	T	T		D	P	P	Y	E
		V	V	T	V	V	V		Y	V		F	P
		T	S	V	S	S	S		F	T		P	V
		V	W	S	W	W	W		P	V		E	T
		S	N	W	N	N	N		E			P	V
		W	S	N	S	S	S		P			V	S
		N	G	S	G	G	G		V			T	W
		S	A	G	A	A	A		T			V	N
		G	L	A	L	L	L		V			S	S
		A	T	L	T	T	T		S			W	G
		L	S	T	S	S	S		W			N	A
		T	G	S	G		G		N			S	L
		S	V	G	V		V		S			G	T
		G	H	V	H		H		G			A	S
		V	T	H	T		T		A			L	G
		H	F	T	F		F		L			T	V
		T	P	F	P		P		T			S	H
		F	A	P	A		A		S			G	T
		P	V	A	V		V		G			V	F
		A	L	V	L		L		V			H	
		V	Q		Q		Q		H			T	
		L										F	
		Q										P	
												A	
												V	

Figure 3

DP-65 or 4-31 gene product  
VSGGSISSGGYYWSWIRQHPGKGLEWIGYIYSGSTIYNFSLKSRVTISVDTSKNQFSLKSSVTAADTAVYYCAR  
CDR1 CDR2

2.1.3 Heavy Chain Protein  
SGPGLVKPSQILSLTCTVSGGSISSGGHYWSWIRQHPGKGLEWIGYIYIGNIYCNFSLKSRVTISVDTSKNQFSLKSSVTAADTAVYYCAR  
CDR1 CDR2  
DSGDYYGIDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
CDR3

**Figure 4**

**A27 Gene Product**  
 EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGGSP  
 CDR1 CDR2 CDR3

**4.1.1 Kappa Chain Protein**  
 QSPGTLSLSPGERATLSCRASQSISSSELAWYQQRPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGTSPWT  
 CDR1 CDR2 CDR3  
 FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK

**4.8.1 Kappa Chain Protein**  
 QSPGTLSLSPGERATLSCRTSMVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGISPET  
 CDR1 CDR2 CDR3  
 FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

**4.14.3 Kappa Chain Protein**  
 GTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGRSPET  
 CDR1 CDR2 CDR3  
 FPGGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

**6.1.1 Kappa Chain Protein**  
 QSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGSSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGISPET  
 CDR1 CDR2 CDR3  
 FPGGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

**4.10.2 Kappa Chain Protein**  
 SPGTLSLSPGERATLSCRASQSISSSELAWYQQKPGQAPRLIYRPSSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGTSPET  
 CDR1 CDR2 CDR3  
 FPGGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ

**4.13.1 Kappa Chain Protein**  
 QSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGASSRATGIPDRFSGSGGTDFLTISRLEPEDFAVYYCQOYGRSPET  
 CDR1 CDR2 CDR3  
 FPGGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKGG

22/48

23/48

**Figure 5****012 Gene Product**

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFRSGSGTDFTLTISSLQPEDFATYYCQOQSYSTPEI  
 CDR1 CDR2 CDR3

**3.1.1 Kappa Chain Protein**

QSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNFLISATISLQSGVPSRFRSGSGTINFILTINSLHPEDFATYYCQOQSYSTPEI  
 CDR1 CDR2 CDR3

FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG

**11.2.1 Kappa Chain Protein**

PSSLSASVGDRVTITCRASQSISSYLDWYQQKPGKAPKLLIYAASSLQSGVPSRFRSGSGTDFTLTISSLQPEDFATYYCQOQSYSTPEI  
 CDR1 CDR2 CDR3

FGPGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV

**11.6.1 Kappa Chain Protein**

TQSPSSLSASVGDRVTITCRASONISRVLNWYQQKPGKAPKFLIYYASILQSGVPSRFRSGSGTDFLTISSLQPEDFATYYCQOQSYSTPEI  
 CDR1 CDR2 CDR3

FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN

**11.7.1 Kappa Chain Protein**

TQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPRVLIYAASSLQSGVPSRFRSGSGTDFLTISSLQPEDFATYYCQOQSYSTPEI  
 CDR1 CDR2 CDR3

FGPGTRVDIERTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAY

24/48

**Figure 6****A10/A26 Gene Product**

EIVLTQSPDFQSVTPKEKVTITCRASQSIGSSILHWYQQKPDQSPKLLIKYASQSEGVPSRFSGSGTDFTLTINSLEAEDAATYYCHQSSSLPQ  
CDR1 CDR2 CDR3

**2.1.3 Kappa Chain Protein**

SPDFQSVTPKEKVTITCRASQSIGSSILHWYQQKPDQSPKLLIKYASQSEGVPSRFSGSGTDFTLTINSLEAEDAATYYCHQSSSLPLT  
CDR1 CDR2 CDR3  
FGGGTKVEIKRTVAAPSVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE



**Figure 7****A17 Gene Product**

DVVMTCQFSLPVTILGQPPASISCRSSOSLVYSDGNTYLNWFQQRPQSPRRLLYKVSINRDSGVDPDRFSGSGGTIDFTLKISRVEAEDVGVYYCMQGTIHWPCDR3

CDR2

CDR1

**12.3.1 Kappa Chain Protein**

PLSLPVTILGQPPASISCRSSOSLVYSDGNTYLNWFQQRPQSPRRLLYKVSINWDSGVDPDRFSGSGGTIDFTLKISRVEAEDVGVYYCMQGSHPPTCDR3

CDR2

CDR1

FGQGITKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

25/48

**Figure 8****A3/A19 Gene Product**

DIVMTQSP<sup>1</sup>SLPVTIPGEPASISCRSSQSL<sup>2</sup>HSNGYN<sup>3</sup>LDWYLQKPGQSPQLLYL<sup>4</sup>GSN<sup>5</sup>RASGV<sup>6</sup>PDFR<sup>7</sup>SGSG<sup>8</sup>GT<sup>9</sup>DF<sup>10</sup>TL<sup>11</sup>KIS<sup>12</sup>RVEA<sup>13</sup>EDV<sup>14</sup>GV<sup>15</sup>YY<sup>16</sup>QM<sup>17</sup>QAL<sup>18</sup>QT<sup>19</sup>E  
CDR1 CDR2 CDR3

**12.9.1 Kappa Chain Protein**

PGEPASISCRSSQSL<sup>2</sup>HSNGYN<sup>3</sup>LDWYLQKPGQSPQLLYL<sup>4</sup>GSN<sup>5</sup>RASGV<sup>6</sup>PDFR<sup>7</sup>SGSG<sup>8</sup>GT<sup>9</sup>DF<sup>10</sup>TL<sup>11</sup>KIS<sup>12</sup>RVEA<sup>13</sup>EDV<sup>14</sup>GV<sup>15</sup>YY<sup>16</sup>QM<sup>17</sup>QAL<sup>18</sup>QT<sup>19</sup>EL<sup>20</sup>I  
CDR1 CDR2 CDR3  
FGG<sup>21</sup>G<sup>22</sup>T<sup>23</sup>K<sup>24</sup>VEI<sup>25</sup>K<sup>26</sup>R<sup>27</sup>T<sup>28</sup>V<sup>29</sup>A<sup>30</sup>PS<sup>31</sup>V<sup>32</sup>FI<sup>33</sup>FP<sup>34</sup>SD<sup>35</sup>EQ<sup>36</sup>L<sup>37</sup>K<sup>38</sup>SG<sup>39</sup>T<sup>40</sup>AS<sup>41</sup>V<sup>42</sup>VC<sup>43</sup>LL<sup>44</sup>NN<sup>45</sup>F<sup>46</sup>Y<sup>47</sup>P<sup>48</sup>R

26/48

27/48

**Figure 9** Amino-terminal amino acid sequence analysis

Hybridoma	Light chain	MW
CT2.1.3	ND	ND
CT3.1.1	NH <sub>2</sub> -DIQMTQSPSSLSASVGDRV	26,119
CT4.1.1	NH <sub>2</sub> -EIVLTQSPGTLSLSPGERAT	23,917
CT4.8.1	NH <sub>2</sub> -EIVLTQSPGTLSLSPGERAT	23,617
CT4.9.1	NH <sub>2</sub> -DIQMTQSPSSVSASVGDRV	23,702
CT4.10.2	NH <sub>2</sub> -TGEFVLTQSPGTLSLSPGER (60%) NH <sub>2</sub> -EFVLTQSPGTLSLSPGERAT (40%)	24,101
CT4.14.3	NH <sub>2</sub> -EIVLTQSPGTLSLSPGERAT	23,770
CT4.13.1	NH <sub>2</sub> -EIVLTQSPGTLSLSPGERAT	23,802
CT6.1.1	NH <sub>2</sub> -EIVLTQSPGTLSLSPGERAT	23,747

Hybridoma	Heavy chain	MW
CT2.1.3	ND	ND
CT3.1.1	NH <sub>2</sub> -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH <sub>2</sub> -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~80%) NH <sub>2</sub> -PEVQF...(minor sequence~20%)	51,813
CT4.1.1	NH <sub>2</sub> -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH <sub>2</sub> -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~65%) NH <sub>2</sub> -PEVQFNWYVD...(minor sequence~35%)	51,502
CT4.8.1	NH <sub>2</sub> -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH <sub>2</sub> -pQ-VQLVESGGGVVQPG(R)SL... (major sequence~60%) NH <sub>2</sub> -PEVQFNWY...(minor sequence~40%)	51,597
CT4.9.1	NH <sub>2</sub> -EVQLLESGGGLVQPGSLRL (free amino terminus)	51,437
CT4.10.2	NH <sub>2</sub> -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH <sub>2</sub> -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~60%) NH <sub>2</sub> -PEVQFNWYVD...(minor sequence~40%)	51,502
CT4.14.3	NH <sub>2</sub> -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH <sub>2</sub> -pQ-VQLVESGGGVVQPGRSL(R)(L)(S) (major sequence~65%) NH <sub>2</sub> -PEVQFNWYV...(minor sequence~35%)	51,293
CT4.13.1	NH <sub>2</sub> -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH <sub>2</sub> -pQ-VQLVESGGGVVQPGRSLRLS (major sequence~75%) NH <sub>2</sub> -PEVQFN...(minor sequence~25%)	51,305
CT6.1.1	NH <sub>2</sub> -Blocked. Following treatment with Pyroglutamate Aminopeptidase: NH <sub>2</sub> -pQ-VQLVESGGGVVEPGRSLRLS* (major sequence~65%) NH <sub>2</sub> -PEVQFNWYVD... (minor sequence~35%)	51,476

\* This heavy chain sequence is similar to the other blocked heavy chain sequences except for a unique Gln->Glu change at position 13.

28/48

Figure 10A

	Conc. (mg/ml)	(Ec1.58)	IEF	SDS-PAGE	SEC	reported MALDI	n-term seq. (c)*	
antibody	reported	observed	observed	(+) b-me	observed	Hc	reported	observed
CT 3.1.1	1.1	1.57	smear	50 & 28 kDa	139,400	51,813	DIQMTQSP	DIQMTQSP
CT 4.1.1	1.54	1.65	smear	50 & 24 kDa	79,900	51,502	EIVLTQSP	EIVLTQSP
CT 4.8.1	1.52	1.54	4 bands	50 & 24 kDa	110,300	51,597	EIVLTQSP	EIVLTQSP
CT 4.10.2	1.29	1.77	4 bands	50 & 25 kDa	107,200	51,502	**	***
CT 4.14.3	1.75	1.65	smear	50 & 24 kDa	82,800	51,293	EIVLTQSP	EIVLTQSP
CT 6.1.1	1.36	1.3	4 bands	50 & 24 kDa	101,100	51,476	EIVLTQSP	EIVLTQSP
* all heavy chains n-terminally blocked (not sequenced in-house)								
** mixed sequences reported: TGEFVLTQSP (60) & EIVLTQSP (40)								
*** mixed sequences observed TGEFVLTQSP (60) & EIVLTQSP (40)								

IOD<sub>280nm</sub> = 0.633 mg/ml  
Ec=1.58

Figure 10D

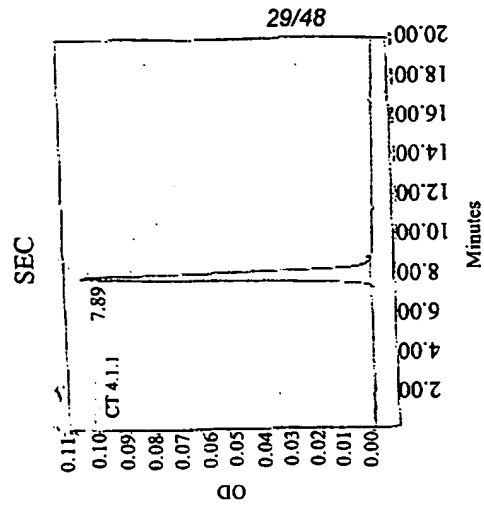


Figure 10C

SDS-PAGE

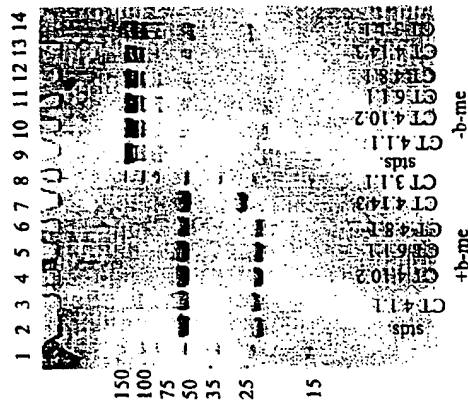
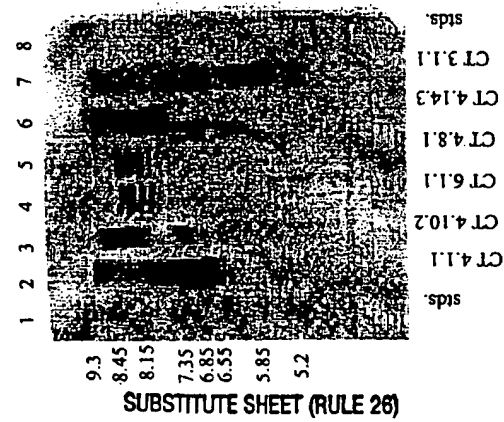
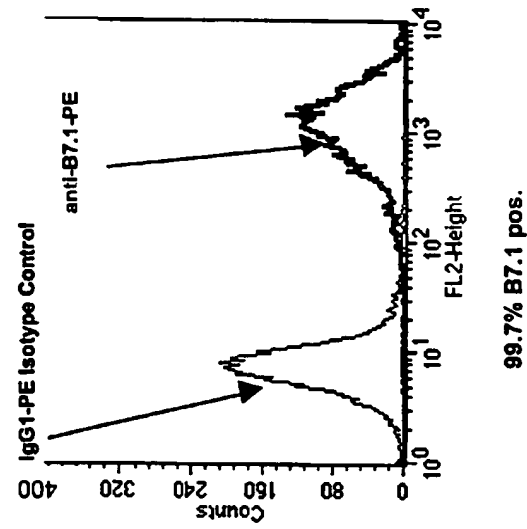


Figure 10B

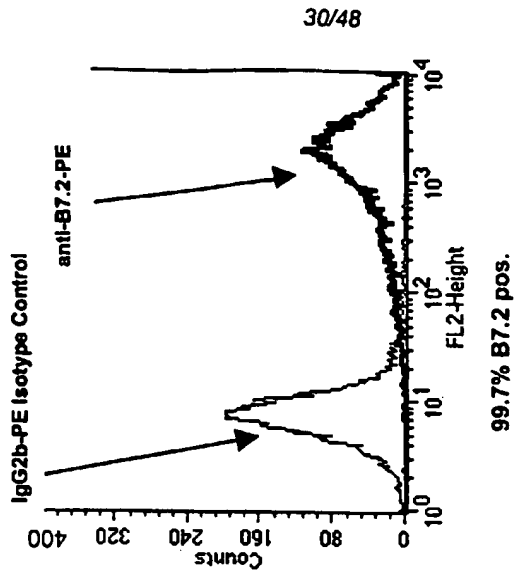
IEF



**Figure 11A**



**Figure 11B**

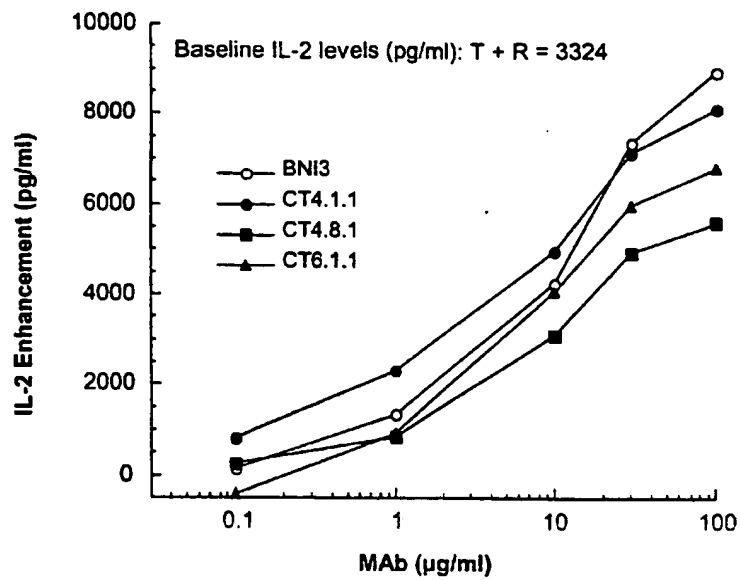


Expression of B7.1 and B7.2 on Raji Cells

31/48

**Figure 12**

**Enhancement of Human T Cell IL-2 Production  
Induced by Anti-CTLA4 XenoMouse MAbs in  
the 72 Hour T Blast / Raji Assay**

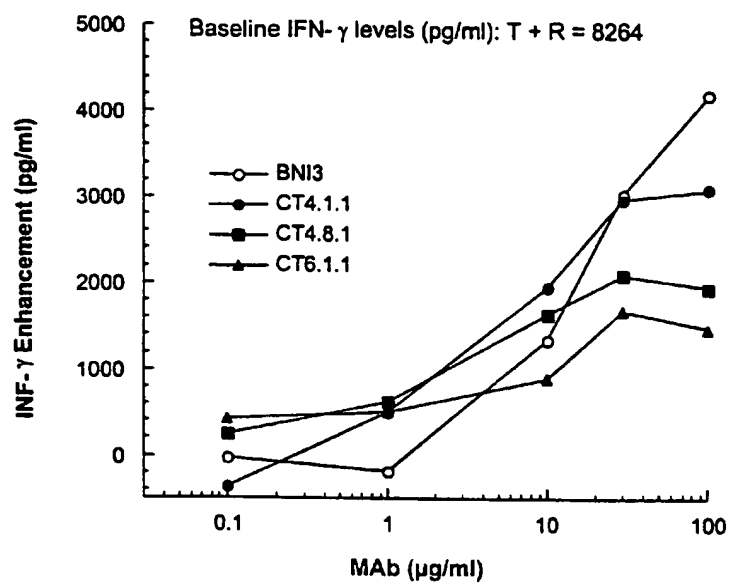


SUBSTITUTE SHEET (RULE 26)

32/48

**Figure 13**

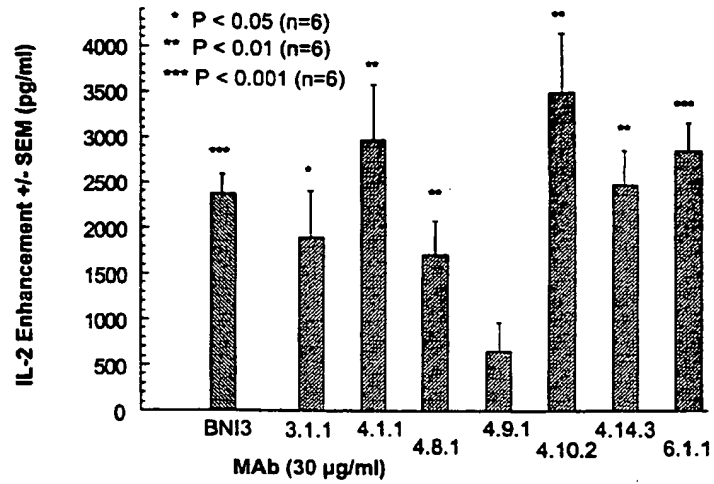
**Enhancement of Human T Cell IFN- $\gamma$  Production  
Induced by Anti-CTLA4 XenoMouse MAbs in  
the 72 Hour T Blast / Raji Assay**





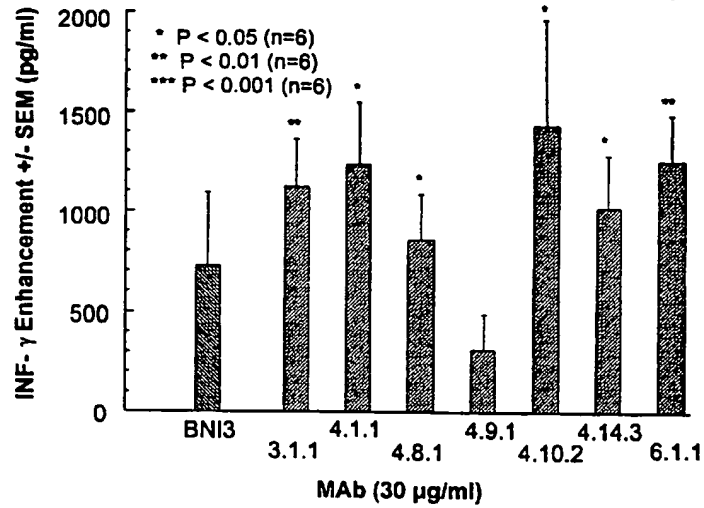
**Figure 14**

**Enhancement of Human T Cell IL-2 Production  
Induced by Anti-CTLA4 XenoMouse MAbs in  
the 72 Hour T Blast / Raji Assay (6 Donors)**  
Baseline IL-2 levels (pg/ml): T + R = 9187, T + R + IgG2a = 9389, T + R + IgG2 = 8509



**Figure 15**

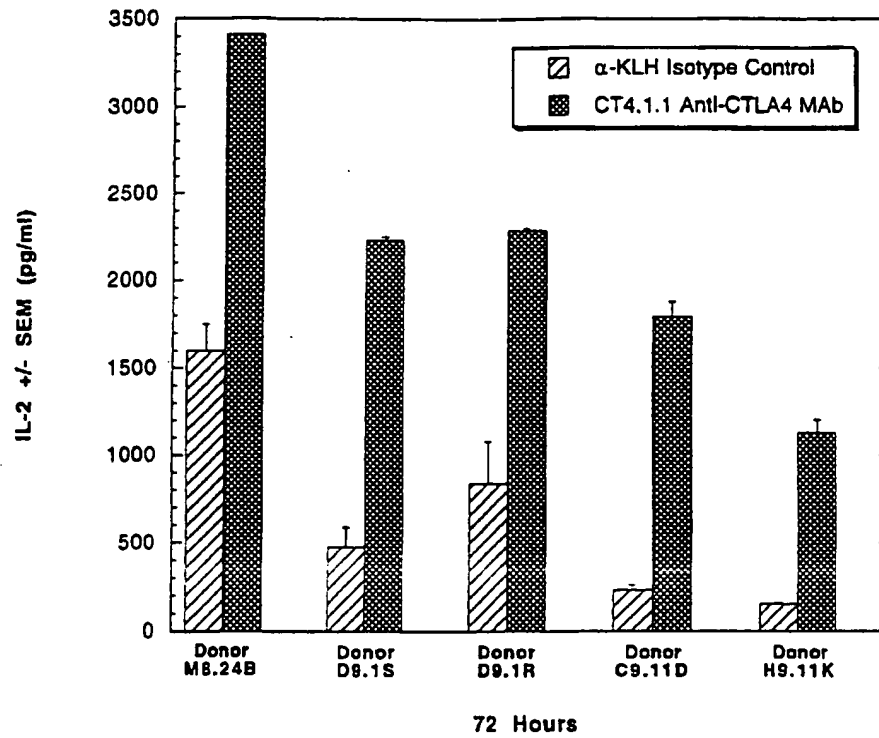
**Enhancement of Human T Cell IFN- $\gamma$  Production  
Induced by Anti-CTLA4 XenoMouse MAbs in  
the 72 Hour T Blast / Raji Assay (6 Donors)**  
Baseline IFN- $\gamma$  levels (pg/ml): T + R = 4780, T + R + IgG2a = 4868, T + R + IgG2 = 4331



35/48

**Figure 16**

Enhancement of IL-2 Production Induced by Anti-CTLA4 MAb  
CT4.1.1 (30  $\mu$ g/ml) Binding to Human PBMC Stimulated with  
SEA (100 ng/ml) Plus Anti-CD3 MAb (60 ng/ml)

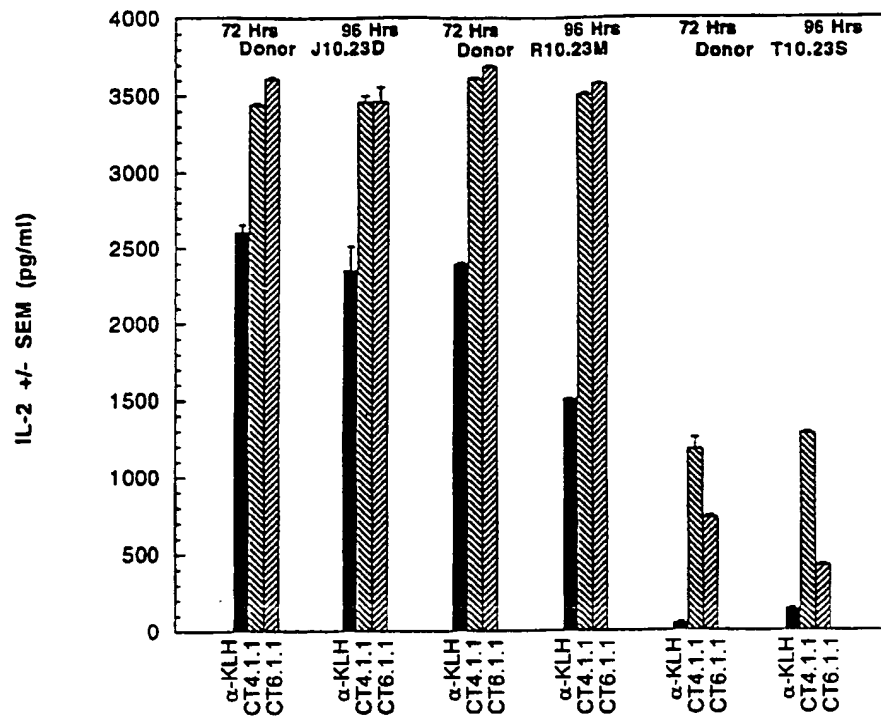


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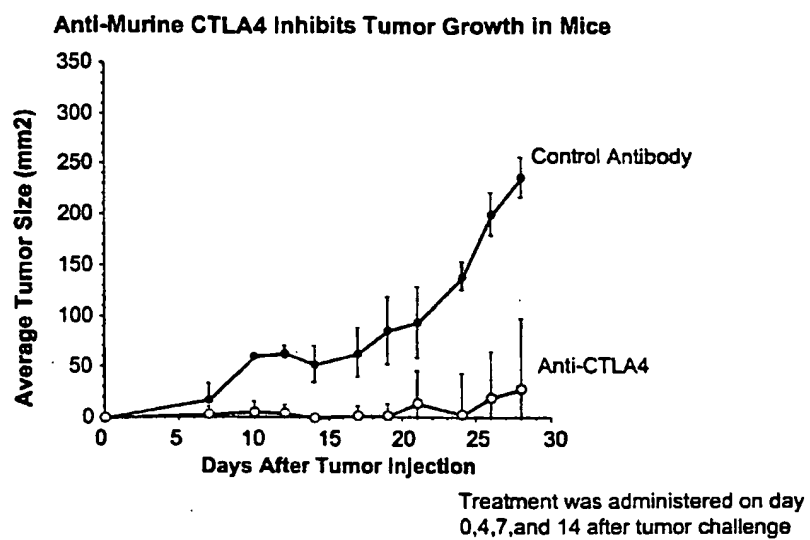
36/48

**Figure 17**

**Enhancement of IL-2 Production Induced by Anti-CTLA4 MAbs  
(30  $\mu$ g/ml) in Human Whole Blood Stimulated with SEA  
(100 ng/ml) Plus Anti-CD3 MAb (60 ng/ml)**



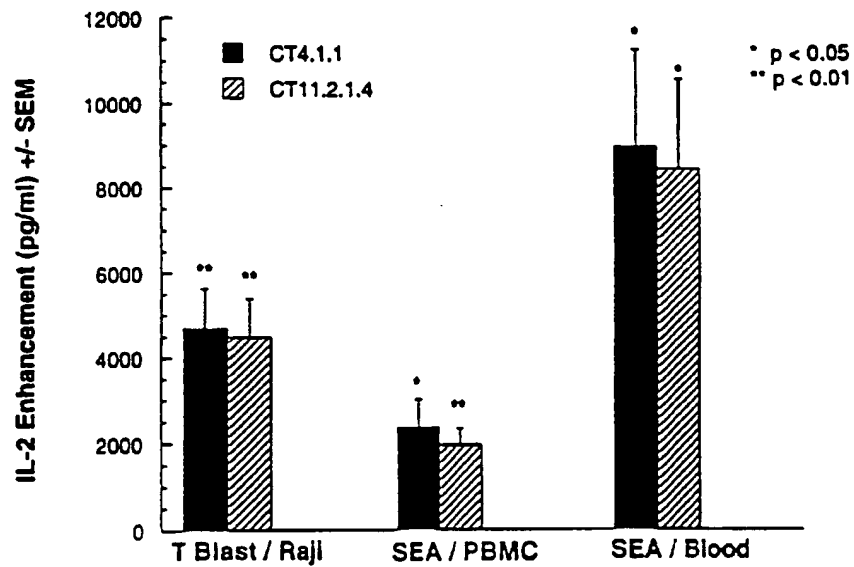
37/48

**Figure 18**

38/48

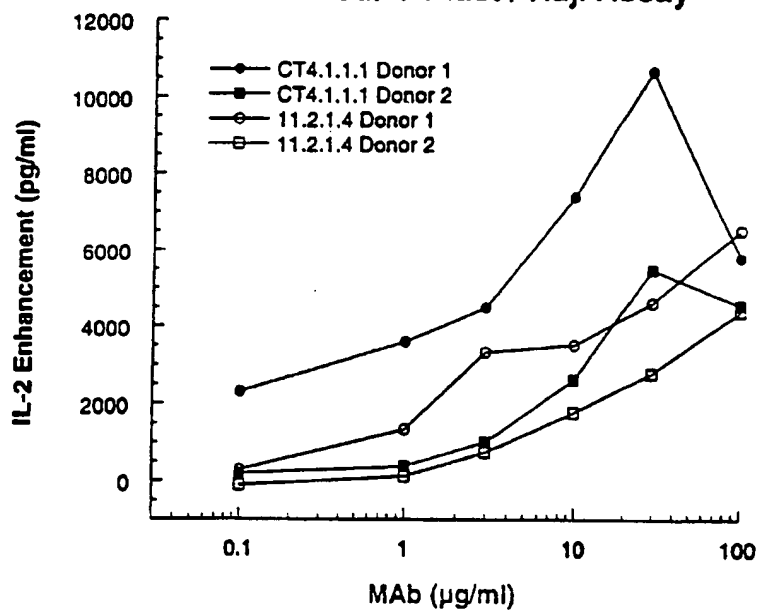
**Figure 19**

Enhancement of IL-2 Production Induced by Anti-CTLA4 MAbs  
(30  $\mu$ g/ml) In the 72 Hour T Blast / Raji and  
Superantigen Assays (6 Donors)



**Figure 20**

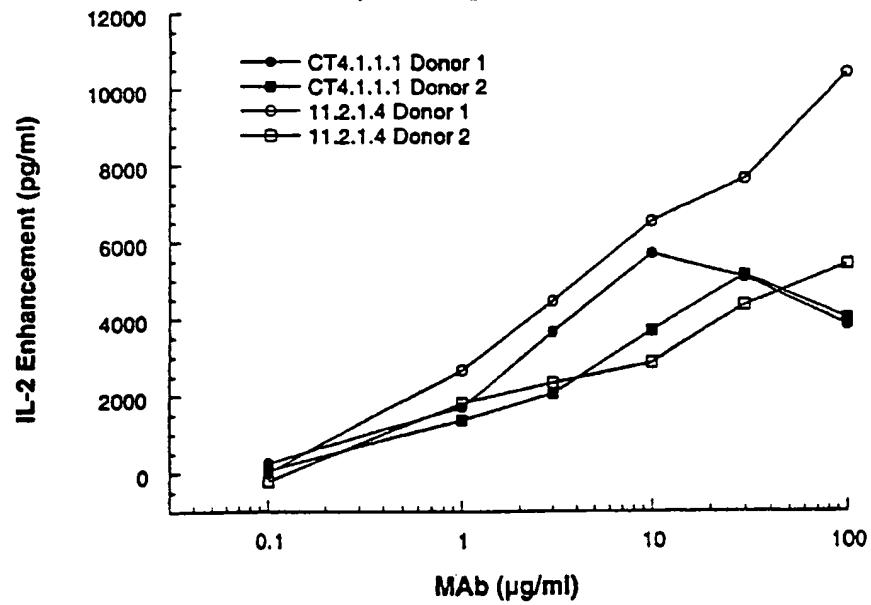
**Enhancement of Human T Cell IL-2 Production  
Induced by Anti-CTLA4 MAbs In  
the 72 Hour T Blast / Raji Assay**



40/48

**Figure 21**

**Enhancement of IL-2 Production Induced by Anti-CTLA4  
MAbs in Whole Blood Stimulated with  
Superantigen (100 ng/ml)**



SUBSTITUTE SHEET (RULE 26)



41/48

- Signal peptides shown in bold and large text
- Open reading frame for genomic clone underlined
- Mutations introduced to make deglycosylated Ab (N294Q) double underlined and large text

**Figure 22A** 4.1.1 IgG2 Heavy Chain cDNA

**ATGGAGTTTGGGCTGAGCTGGGTTTCTCGTTGCTCTTTTAAGAG**  
**GTGTCCAGTGT**CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC  
CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTACCTTCAGTAGC  
CATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC  
AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC  
GATTACCATCTCCAGAGACAATCCAAGAACACGCTGTTTCTGCAAATGAAC  
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTT  
CGGTCTTTTGACTACTGGGGCCAGGGAACCTGGTCACCGTCTCCTCAGCCT  
CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC  
GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT  
GACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAG  
CTGTCTTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC  
TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCAG  
CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCAC  
CGTGCCCGACACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAA  
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACGTGCGTGGTGGT  
GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG  
TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG  
TTCCGTGTGGTCAGCGTCTCACCCTGTGTGCACCAGGACTGGCTGAACGGCAA  
GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCAGCCCCCATCGAGAAAA  
CCATCTCCAAAACCAAAGGGCAGCCCGAGAACCAAGGTGTACACCTGCC  
CCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAA  
AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG  
AGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC  
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT  
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC  
TCTCCCTGTCTCCGGTAAATGA (SEQ ID NO:53)

42/48

**Figure 22B** 4.1.1 IgG2 Heavy Chain Genomic DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG  
 GTGTCCAGTGTTCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC  
 CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTACCTTCAGTAGC  
 CATGGCATGCACTGGGTCCGCCAGGCTCCAGGCCAAGGGGCTGGAGTGGGTGGC  
 AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC  
 GATTTCACCATCTCCAGAGACAATCCAAAGAACCGCTGTTTCTGCAAAATGAAC  
 AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTT  
 CGGTCTCTTTGACTACTGGGGCCAGGGAACCTGGTCAACCGTCTCCTCAGCTA  
 GCACCAAGGGCCCATCGGTCTTCCCTCTGGCGCCTGTCTCCAGGAGCACCTCC  
 GAGAGCACAGCGGCCCTGGGCTGCTGGTCAAGGACTACTTCCCGAACCCTG  
 GACCGTGTCTGGAACTCAGGCGCTCTGACCGCGCGTGCACACTTCCCGAG  
 CTGTCTTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTACCGTGGCC  
 TCCAGCAACTTCCGCCACCCAGACCTACACCTGCACGTAAGTACACAAGCCAG  
 CAACACCAAGGTGGACAAGACAGTTGGTGAGAGGCCAGCTCAGGGAGGGAGGG  
 TGTCTGTGGAAGCCAGGCTCAGCCCTCCTGCCCTGGACGCACCCCGGCTGTGC  
 AGCCCCAGCCCAGGGCAGCAAGGCAGGCCCATCTGTCTCCTCACCCGGAGGC  
 CTCTGCCGCCCCCACTCATGCTCAGGAGAGGGTCTTCTGGCTTTTTCACCA  
 GGCTCCAGGCAGGCACAGGCTGGGTGCCCTACCCAGGCCCTTCACACACAG  
 GGGCAGGTGCTTGGCTCAGACCTGCCAAAAGCCATATCCGGGAGGACCTGCC  
 CCTGACCTAAGCCGACCCCAAAGGCCAACTGTCCACTCCCTCAGCTCGGACA  
 CCTTCTCTCCTCCAGATCCGAGTAACTCCCAATCTTCTCTCTGCAGAGCGCA  
 AATGTTGTGTCGAGTGCCCAACCGTGCCAGGTAAGCCAGCCCAGGCCTCGCCC  
 TCCAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG  
 ACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAAGCGTCT  
 CACCGTTGTGCACCGAGTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCT  
 CCAACAAAGGCCTCCAGCCCCATCGAGAAAACCATCTCCAAAACCAAGGT  
 GGGACCCGCGGGTATGAGGGCCACATGGACAGAGCCGGCTCGGCCCACCT  
 CTGCCCTGGGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGGCAGCCCCGA  
 GAACACAGGTGTACACCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA  
 GGTACAGCTGACCTGCTGGTCAAGGCTTCTACCCAGCGACATCGCCGTGG  
 AGTGGGAGAGCAATGGGCAGCCGAGAACAACTACAAAGACCACACCTCCCATG  
 CTGGACTCCGACCGCTCTCTCTCTCTCAGCAAGCTCACCCTGGACAAGAG  
 CAGGTGGCAGCAGGGGAACGTTCTCTCATGCTCCGTGATGCATGAGGCTCTGC  
 ACAACCACTACACGCAGAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ  
 ID NO: 54)

**Figure 22C** 4.1.1 IgG2 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGSRSLRLSCVASGFTFSS  
 HGMHWVRQAPGKGLEWVAIVYDGRNKYYADSVKGRFTISRDNKNTLFLQMN  
 SLRAEDTAVYYCARGGHFGPFDYWGQGLVTVSSASTKGPSVFPLAPCSRSTS  
 ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVF  
 SSNFGTQTYTCNVDHKPSNTKVDKTVKCCVBCPPCPAPPVAGPSVFLFPPK  
 PKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST  
 FRVSVLTVVHQDNLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLF  
 PSRERMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFF  
 LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID  
 NO: 63)

SUBSTITUTE SHEET (RULE 26)

43/48

**Figure 22D** 4.1.1 IgG2 Heavy Chain cDNA N2940

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG  
 GTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGAGGCGTGGTCCAGC  
 CTGGGAGGTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTACCTTCAGTAGC  
 CATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC  
 AGTTATATGGTATGATGGAAGAAATAAATACTATGCAGACTCCGTGAAGGGCC  
 GATTACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAAC  
 AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGGTCACTT  
 CGGTCTTTTGTACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCT  
 CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCTGTCCAGGAGCACCTCC  
 GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT  
 GACGGTGTGCTGGAACCTCAGGCGCTCTGACCAGCGGCTGCACACCTTCCCAG  
 CTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC  
 TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCGAG  
 CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCAC  
 CGTGCCCGCAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAA  
 CCCAAGGACACCCCTCATGATCTCCCGACCCCTGAGGTACGTCGCTGGTGGT  
 GGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG  
 TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCCAAAGCAGC  
 TTCCGTGTGGTCAGCGTCTCACCCTTGTGACCAAGGACTGGCTGAACGGCAA  
 GGAGTACAAGTGCAAGGTCTCCAACAAAGGCTCCAGCCCCATCGASAAAA  
 CCATCTCCAAACCAAAGGGCAGCCCCGAGAACACAGGTGTACACCTGCCC  
 CCATCCCGGGAGGAGATCACCAAGAACAGGTGACGCTGACCTGCCTGGTCAA  
 AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG  
 AGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC  
 CTCTACAGCAAGCTCACCCTCGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT  
 CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC  
 TCTCCTGTCTCCGGGTAAATGA (SEQ ID NO:55)

**Figure 22E** 4.1.1 IgG2 Heavy Chain Protein N2940

MEFGLSWVFLVALLRGVQCQVQLVESGGGVQPGRSLRLSCVASGFTFSS  
 HGMHWVRQAPGKLEWVAVIWDGRNKYYADSVKGRFTISRDNKNTLFLQMN  
 SLRAEDTAVYYCARGGHFGPFDYWQGTLVTVSSASTKGPSVPPLAPCSRSTS  
 ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP  
 SSNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPPCPAPPVAGPSVFLFPPK  
 PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQST  
 FRVVSVLTVVHQDLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLF  
 PSREBMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSGGSFF  
 LYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID  
 NO:64)

44/48

**Figure 22F** 4.1.1 Kappa Chain DNA

ATGGAAACCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC  
CAGATACCACCGGAGAAATTGTGTGACGCAGTCTCCAGGCACCCTGTCTT  
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTATTAGC  
AGCAGCTTCTTAGCCTGGTACCAGCAGAGACCTGGCCAGGCTCCAGGCTCCT  
CATCTATGGTGCATCCAGCAGGGCCACTGGCATCCAGACAGGTTTCAGTGGCA  
GTGGGTCTGGGACAGACTTCACTCTACCATCAGCAGACTGGAGCCTGAAGAT  
TTTGCACTGTATTACTGTCAGCAGTATGGTACCTCACCCCTGGACGTTCCGGCCA  
AGGGACCAAGGTGGAATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCT  
TCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTG  
CTGAATAACTTCTATCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGC  
CCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA  
GCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAA  
CACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC  
AAAGAGCTTCAACAGGGGAGAGTGTAG (SEQ ID NO:56)

**Figure 22G** 4.1.1 Kappa Chain Protein

METPAQLLFLLLLLWLPDTTGEIVLTQSPGTLSPGERATLSCRASQIS  
SSFLAWYQORPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPED  
FAVYYCQQYGTSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC  
LNNFYPRFAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSLTLSKADYEK  
HKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:65)

**Figure 22H** 4.8.1 Heavy Chain DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG  
GTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGC  
CTGGGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTACCTTCAGTAAC  
TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGTGGC  
AGTTATATGGTATGATGGAAGTAATAAACTATGGAGACTCCGTGAAGGGCC  
GATTACCATCTCCAGTGACAATTCCAAGAACACGCTGTATCTGCAAAATGAAC  
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGGAGAGAGACT  
GGGGTCTCTACTTTGACTACTGGGGCCAGGGAACCCCTGGTCACCGTCTCCTCAG  
CCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACC  
TCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACC  
GGTGACGGTGTCTGTGGAATCAGGCGCTCTGACCAGCGGCTGCACACCTTCC  
CAGCTGTCTTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG  
CCCTCCAGCAACTTCGGCACCCAGACCTACCTGCAACGTAGATCACAAGCC  
CAGCAACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTGAGTGCC  
CACCGTGCCAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCA  
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACGTGCGTGGT  
GGTGGAGCTGAGCCACGAAGACCCGAGGTCCAGTTCAACTGGTACGTGGACG  
GCGTGGAGGTGCATAATGCCAAGCAAAGCCACGGGAGGAGCAGTTCAACAGC  
ACGTTCCGTGTGGTCAAGCTCTCACCCTGTGCAACAGGACTGGCTGAACGG  
CAAGGAGTACAAGTGAAGGTCTCCAACAAGGCCTCCAGCCCCCATCGAGA  
AAACCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCTG  
CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCTGGT  
CAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC  
CGGAGAACAATAAGACACACCTCCCATGCTGGACTCCGACGGCTCCTTC  
TTCCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGAACGT  
CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGA  
GCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:57)

45/48

**Figure 22I** 4.8.1 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVVQPGRSRLRLSCTASGFTFSN  
YGMHWVRQAPGKGLEWVAVIWDGSKNHYGDSVKGRFTISSDNSKNTLYLQMN  
SLRAEDTAVYYCARGERLGSYFDYWGQGLVTVSSASTKGPSVFPLAPCSRST  
SESTAALGCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSLSSVTV  
PSSNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPPCPAPPVAGPSVFLFPP  
KPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS  
TFRVSVSLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL  
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSGGSF  
FLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK (SEQ ID  
NO:66)

**Figure 22J** 4.8.1 Kappa Chain DNA

ATGGAAACCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC  
CAGATACCAACCGGAGAAATTGTGTGACGCAGTCTCCAGGCACCCTGTCTT  
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGACCAGTGTAGCAGCAGT  
TACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGCTCCTCATCTA  
TGGTGATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCACTGGCAGTGGGT  
CTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTGCA  
GTCTATTACTGTGACAGTATGGCATCTCACCTTCACTTTCGGCGGAGGGAC  
CAAGGTGGAGATCAAGCGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGC  
CATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAAT  
AACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCCA  
ATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCT  
ACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAA  
GTCTACGCCTGCCAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG  
CTTCAACAGGGGAGAGTGTTAG (SEQ ID NO:58)

**Figure 22K** 4.8.1 Kappa Chain Protein

METPAQLLFLLLLWLPDTTGEIVLTQSPFTLSLSPGERATLSCRTSVSSS  
YLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA  
VYYCQQYGISPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN  
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHK  
VYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO:67)

46/48

**Figure 22L** 6.1.1 Heavy Chain DNA

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAGAG  
 GTGTCCAGTGT CAGGTGCAGCTGGTGGAGTCTGGGGAGGCGTGGTCGAGC  
 CTGGGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTACCTTCAGTAGT  
 TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC  
 AGTTATATGGTATGATGGAAGCAATAAACAATATGCAGACTCCGCGAAGGGCC  
 GATTCAACCATCTCCAGAGACAAATCCAAGAACACGCTGTATCTGCAAATGAAC  
 AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGCCGACTGTCT  
 GGGTTACTTTGACTACTGGGGCCAGGGAACCTGGTCACCGTCTCCTCAGCCT  
 CCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGTCCAGGAGCACCTCC  
 GAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT  
 GACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCCAG  
 CTGTCTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC  
 TCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAG  
 CAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCAC  
 CGTGCCCGAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAA  
 CCCAAGGACACCTCATGATCTCCCGACCCCTGAGGTACGTGCGTGGTGGT  
 GGAGTGGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCG  
 TGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG  
 TTCCGTGTGGTCAAGCTCCTCACCGTTGTGACACGAGTGGCTGAACGGCAA  
 GGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCAGCCCCATCGAGAAAA  
 CCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCC  
 CCATCCCGGGAGGAGATGACCAAGAACCAGCTCAGCCTGACCTGCCTGGTCAA  
 AGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG  
 AGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC  
 CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT  
 CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC  
 TCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:59)

**Figure 22M** 6.1.1 Heavy Chain Protein

MEFGLSWVFLVALLRGVQCQVQLVESGGGVPEGRSLRLSCTASGFTFSS  
 YGMHWVRQAPGKLEWVAVIWDGSKHYADSAKGRFTISRDNKNTLYLQMN  
 SLRAEDTAVYYCARAGLLGYFDYWQGTTLTVSSASTKGPSVFPLAPCSRSTS  
 ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP  
 SSNFGTQTYTCNVDPKPSNTKVDKTVKCCVECPPEPPVAGPSVFLFPPK  
 PKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST  
 FRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLT  
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMLDSGDSFF  
 LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID  
 NO:68)

47/48

**Figure 22N** 6.1.1 Kappa Chain DNA

ATGGAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCC  
CAGATACCACCGGAGAAATTGTGTTGACGAGTCTCCAGGCACCTGTCTT  
TGTCTCCAGGGGAAAGAGCCACCCTCTCCTGTAGGGCCAGTCAAAGTGTTAGC  
AGCTACTTAGCCTGGTACCAACAGAAACCTGGCCAGGCTCCAGGCCCTCAT  
CTATGGTGTATCCAGCAGGGCCACTGGCATCCAGACAGGTTCAAGTGGCAGTG  
GGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT  
GCAGTGTATTACTGTGTCAGCAGTATGGTATCTCACCATTCACTTTCGGCCCTGG  
GACCAAAGTGGATATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC  
CGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTG  
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCT  
CCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA  
CCTACAGCCTCAGCAGCACCTTGACGCTGAGCAAAGCAGACTACGAGAAACAC  
AAAGTCTACGCCTGCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCACAAA  
GAGCTTCAACAGGGGAGAGTGTAG (SEQ ID NO:60)

**Figure 22O** 6.1.1 Kappa Chain Protein

METPAQLLFLLLLWLPDITGEIVLTQSPGTLSPGERATLSCRASQSVS  
SYLAWYQQKPGQAPRPLIYGVSSRATGIPDRFSGSGSGTDFTLTISRLEPEDF  
AVYYCQQYGISPTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL  
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKH  
KVYACEVTHQGLSSPVTIKSFNRGEC (SEQ ID NO:69)

**Figure 22P** 11.2.1 IgG2 Heavy Chain DNA:

ATGGAGTTTGGGCTGAGCTGGGTTTTCTCCTCGTTGCTCTTTTAAGAG  
GTGTCCAGTGTGTCAGGTGAGCTGGTGGAGTCTGGGGAGGCGTGGTCCAGC  
CTGGGAGGTCCCTGAGACTCTCCTGTGTCAGCGTCTGGATTACCTTCAGTAGC  
TATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGC  
AGTTATATGGTATGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCC  
GATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAAC  
AGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCGAGGGG  
AGCTACCTTTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCA  
CGGTCAACGCTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCG  
CCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAA  
GGACTACTTCCCCGAACCGGTGACGGTGTGCTGGAACCTCAGGCGCTCTGACCA  
GCGGCGTGACACCTTCCCAGCTGTCTTACAGTCTCAGGACTCTACTCCCTC  
AGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTG  
CAACGTAGATCACAAGCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCA  
AATGTTGTGTGAGTGGCCACCGTGCCCGAGCACCACCTGTGGCAGGACCGTCA  
GTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCC  
TGAGGTCAAGTGCCTGGTGGTGGAGCTGAGCCACGAAGACCCCGAGGTCCAGT  
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGG  
GAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAAGCTCCTCACCCTTGTGCA  
CCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCC  
TCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAGGGCAGCCCCGAGAA  
CCACAGGTGTACACCTGCCCCCATCCCGGAGGAGATGACCAAGAACCAGGT  
CAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGT  
GGGAGAGCAATGGGCAGCCGAGACAACACTACAAGACCACACCTCCCATGCTG  
GACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAAGAGCAG  
GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACA  
ACCACTACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGA (SEQ ID  
NO:61)

**Figure 22Q** 11.2.1 IgG2 Heavy Chain Protein:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWIY  
DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDPRGATLY  
YYYYGMDVWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFP  
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNVDH  
KPSNTKVDKTVKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTC  
VVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHODWL  
NGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTC  
LVKGFYPSDIAVEWESNGQPENNYKTPPMLDSDGSFFLYSKLTVDKSRWQQG  
NVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:70)

**Figure 22R** 11.2.1 IgG2 Kappa Chain DNA:

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCT  
GGCTCCGAGGTGCCAGATGTGACATCCAGATGACCCAGTCTCCATCCTCC  
CTGTCTGCACTCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAG  
CATTAAACAGCTATTTAGATTGGTATCAGCAGAAACCAGGGAAAGCCCCATAAC  
TCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCACT  
GGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGA  
AGATTTTGCAACTTACTACTGTCAACAGTATTACAGTACTCCATTCACTTTCG  
GCCCTGGGACCAAAGTGGAAATCAAACGAACTGTGGCTGCACCATCTGTCTTC  
ATCTTCCC GCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTG  
CCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGGAAGGTGGATA  
ACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAG  
GACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGA  
GAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCCG  
TCACAAAGAGCTTCAACAGGGGAGAGTGTTAGTGA (SEQ ID NO:62)

**Figure 22S** 11.2.1 IgG2 Kappa Chain Protein:

DIQMTQSPSSLSASVGDRTITCRASQINSYLDWYQQKPGKAPKLLIYAASS  
LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQYYSTPFTFGPGTKVEI  
KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNS  
QESVTEQDSKDSSTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG  
EC (SEQ ID NO:71)



# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/30895

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/28 C12N5/08 G01N33/53 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 42752 A (BRIGHAM AND WOMEN'S HOSPITAL) 1 October 1998 (1998-10-01) claims	1-56
A	WO 95 33770 A (REPLIGEN CORPORATION ET AL.) 14 December 1995 (1995-12-14) page 17, line 19 - line 29 claim 12	1-56
A	A. JAKOBOVITS: "The long-awaited magic bullets: therapeutic human monoclonal antibodies from transgenic mice." EXPERT OPINION ON INVESTIGATIONAL DRUGS, vol. 7, no. 4, April 1998 (1998-04), pages 607-614, XP002084509 London, GB abstract figures 1,3	1-56

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Date of the actual completion of the international search

24 May 2000

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 33735 A (CELL GENESYS, INC.) 31 October 1996 (1996-10-31) page 14, line 19 - line 28 claim 20	1-56
A	WO 98 50433 A (ABGENIX, INC.) 12 November 1998 (1998-11-12) examples claims	1-56
A	P. MARRACK ET AL.: "The staphylococcal enterotoxins and their relatives." SCIENCE, vol. 248, no. 4956, 11 May 1990 (1990-05-11), pages 705-711, XP002138564 New York, NY, USA the whole document	86-104

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information on patent family members

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WO 9850433 A	12-11-1998	AU 7287098 A EP 0979246 A	27-11-1998 16-02-2000



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US99/30895 <b>(22) International Filing Date:</b> 23 December 1999 (23.12.1999) <b>(30) Priority Data:</b> 60/113,647 23 December 1998 (23.12.1998) US <b>(60) Parent Application or Grant</b> PFIZER, INC. [/]; O. ABGENIX, INC. [/]; O. HANSON, Douglas, Charles [/]; O. NEVEU, Mark, Joseph [/]; O. MUELLER, Eileen, Elliott [/]; O. HANKE, Jeffrey, Herbert [/]; O. GILMAN, Steven, Christopher [/]; O. DAVIS, C., Geoffrey [/]; O. CORVALAN, Jose, Ramon [/]; O. HANSON, Douglas, Charles [/]; O. NEVEU, Mark, Joseph [/]; O. MUELLER, Eileen, Elliott [/]; O. HANKE, Jeffrey, Herbert [/]; O. GILMAN, Steven, Christopher [/]; O. DAVIS, C., Geoffrey [/]; O. CORVALAN, Jose, Ramon [/]; O. HARE, Christopher, A. ; O.		<b>Published</b>
<b>(54) Title: HUMAN MONOCLONAL ANTIBODIES TO CTLA-4</b> <b>(54) Titre: ANTICORPS MONOCLONAUX HUMAINS DIRIGES CONTRE L'ANTIGENE CTLA-4</b>  <b>(57) Abstract</b> <p>In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Also provided is a cell culture system for assaying T cell stimulation.</p> <b>(57) Abrégé</b> <p>La présente invention concerne des anticorps monoclonaux entièrement humains dirigés contre l'antigène CTLA-4. L'invention concerne également des séquences nucléotidiques codant des molécules d'immunoglobuline à chaînes lourdes et légères, des séquences d'acides aminés contenant des molécules d'immunoglobuline à chaînes lourdes et légères et notamment des séquences de chaînes lourdes et légères contiguës, couvrant les régions déterminant la complémentarité (CDR), spécifiquement depuis l'intérieur de FR1 et/ou CDR1 jusqu'à CDR3 et/ou l'intérieur de FR4. En outre, l'invention concerne des anticorps présentant des propriétés de liaison similaires et des anticorps (ou autres antagonistes) ayant une fonctionnalité similaire en tant qu'anticorps.</p>		

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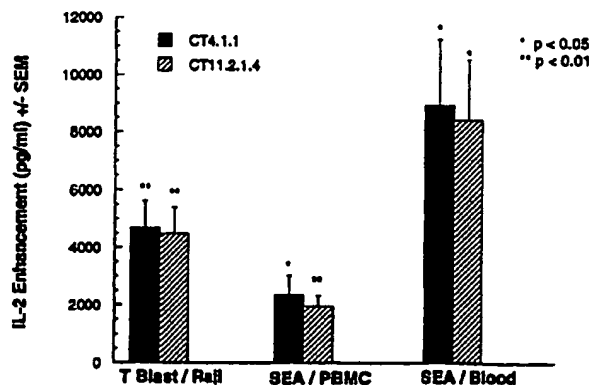


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US99/30895 (22) International Filing Date: 23 December 1999 (23.12.99) (30) Priority Data: 60/113,647 23 December 1998 (23.12.98) US (71) Applicants (for all designated States except US): PFIZER, INC. [US/US]; Eastpoint Road, Groton, CT 06340 (US). AB- GENIX, INC. [US/US]; 7601 Dumbarton Circle, Fremont, CA 94555 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HANSON, Douglas, Charles [US/US]; 3 Acom Drive, Niantic, CT 06357 (US). NEVEU, Mark, Joseph [US/US]; 18 Greenbriar Court, Mystic, CT 06355 (US). MUELLER, Eileen, Elliott [US/US]; 4 Butterwick Lane, Old Lyme, CT 06371 (US). HANKE, Jeffrey, Herbert [US/US]; 1 Jefferson Circle, Reading, MA 01867 (US). GILMAN, Steven, Christopher [US/US]; 118 Sill Lane, Old Lyme, CT 06371 (US). DAVIS, C., Geoffrey [US/US]; 1132 Vancouver Avenue, Burlingame, CA 94010 (US). CORVALAN, Jose, Ramon [CL/US]; 125 Williams Lane, Foster City, CA 94010 (US).		(74) Agent: HARE, Christopher, A.; Abgenix, Inc., 7601 Dumbarton Circle, Fremont, CA 94555 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
		Published With international search report. (88) Date of publication of the international search report: 14 September 2000 (14.09.00)	

(54) Title: HUMAN MONOCLONAL ANTIBODIES TO CTLA-4

Enhancement of IL-2 Production Induced by Anti-CTLA4 MAb  
(30 µg/ml) in the 72 Hour T Blast / Raji and  
Superantigen Assays (6 Donors)



(57) Abstract

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Also provided is a cell culture system for assaying T cell stimulation.

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CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

# INTERNATIONAL SEARCH REPORT

Int'l Application No.  
PCT/US 99/30895

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC 7	C07K16/28 C12N5/08	G01N33/53 G01N33/68
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 7 C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 42752 A (BRIGHAM AND WOMEN'S HOSPITAL) 1 October 1998 (1998-10-01) claims	1-56
A	WO 95 33770 A (REPLIGEN CORPORATION ET AL.) 14 December 1995 (1995-12-14) page 17, line 19 - line 29 claim 12	1-56
A	A. JAKOBOVITS: "The long-awaited magic bullets: therapeutic human monoclonal antibodies from transgenic mice." EXPERT OPINION ON INVESTIGATIONAL DRUGS, vol. 7, no. 4, April 1998 (1998-04), pages 607-614, XP002084509 London, GB abstract figures 1,3	1-56
-/-		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
24 May 2000		13/06/2000
Name and mailing address of the ISA European Patent Office, P.O. 5518 Patentaan 2 NL - 2280 MV Rijswijk Tel. (+31-70) 340-2040, Tlx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  Nooij, F

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# INTERNATIONAL SEARCH REPORT

Inter- national Application No  
PCT/US 99/30895

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 33735 A (CELL GENESYS, INC.) 31 October 1996 (1996-10-31) page 14, line 19 - line 28 claim 20	1-56
A	WO 98 50433 A (ABGENIX, INC.) 12 November 1998 (1998-11-12) examples claims	1-56
A	P. MARRACK ET AL.: "The staphylococcal enterotoxins and their relatives." SCIENCE, vol. 248, no. 4956, 11 May 1990 (1990-05-11), pages 705-711, XPO02138564 New York, NY, USA the whole document	86-104



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information on patent family members

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